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(54) Gene conferring resistance to Phytophthera infestans (late-blight) in Solanaceae

(57) The invention relates to the field of plant diseases, in particular to oomycete infections such as late blight, a disease of major importance to production of *Solanacae* such as potato and tomato cultivars. The invention provides a method for providing a plant or its progeny with resistance against an oomycete infection comprising providing said plant or part thereof with a gene or functional fragment thereof comprising a nucleic acid, said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* with resistance against an oomycete fungus.

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Description

[0001] The invention relates to the field of plant diseases.

[0002] Late blight, caused by the oomycete pathogen *Phytophthora infestans* is world-wide the most destructive disease for potato cultivation. The disease also threatens the tomato crop. The urgency of obtaining resistant cultivars has intensified as more virulent, crop-specialised and pesticide resistant strains of the pathogen are rapidly emerging.

[0003] A way to prevent crop failures or reduced yields is the application of fungicides that prevent or cure an infection by *P. infestans*. However, the application of crop protectants is widely considered to be a burden for the environment. Thus, in several Western countries, legislation is becoming more restrictive and partly prohibitive to the application of specific fungicides, making chemical control of the disease more difficult. An alternative approach is the use of cultivars that harbour partial or complete resistance to late blight. Two types of resistance to late blight have been described and used in potato breeding. One kind is conferred by a series of major, dominant genes that render the host incompatible with specific races of the pathogen (race specific resistance). Eleven such *R* genes (*R1-R11*) have been identified and are believed to have originated in the wild potato species *Solanum demissum,* which is native to Mexico, where the greatest genetic variation of the pathogen is found. Several of these *R* genes have been mapped on the genetic map of potato. *R1* and *R2* are located on chromosomes 5 and 4, respectively. *R3, R6* and *R7* are located on chromosome 11. Unknown *R* genes conferring race specific resistance to late blight have also been described in *S. tuberosum* ssp. *andigena* and *S. berthaultii*. Because of the high level of resistance and ease of transfer, many cultivars contain *S. demissum* derived resistance. Unfortunately, *S. demissum* derived race specific resistance, although nearly complete, is not durable. Once newly bred cultivars are grown on larger scale in commercial fields, new virulences emerge in *P. infestans* that render the pathogen able to overcome the introgressed resistance. The second type of resistance, often quantitative in nature, is race non-specific and is thought to be more durable. Race non-specific resistance to late blight can be found in several Mexican and Middle and South American *Solanum* species.

[0004] Diploid *S. bulbocastanum* from Mexico and Guatemala is one of the tuber bearing species that is known for its race non-specific resistance to late blight. Despite differences in endosperm balance numbers, introgression of the *S. bulbocastanum* resistance trait has been successful. Ploidy manipulations and a series of tedious bridge crosses has resulted in *S. bulbocastanum* derived, *P. infestans* resistant germplasm. However, almost 40 years after the first crosses and intense and continuous breeding efforts by potato breeders in the Netherlands with this germplasm, late blight resistant cultivars still remain to be introduced on the market. Successful production of somatic hybrids of *S. bulbocastanum* and *S. tuberosum* has also been reported. Some of these hybrids and backcrossed germplasm were found to be highly resistant to late blight, even under extreme disease pressure. Despite reports of suppression of recombination, resistance in the backcrossed material appeared to be on chromosome 8 within an approximately 6 cM interval between the RFLP markers CP53 and CT64. A CAPS marker derived from the tomato RFLP probe CT88 cosegregated with resistance. Suppression of recombination between the *S. bulbocastanum* and *S. tuberosum* chromosomes forms a potential obstacle for successful reconstitution of the recurrent cultivated potato germplasm to a level that could meet the standards for newly bred potato cultivars. Isolation of the genes that code for resistance found in *S. bulbocastanum* and subsequent transformation of existing cultivars with these genes, would be a much more straight forward and quicker approach when compared to introgression breeding.

[0005] The cloning and molecular characterisation of numerous plant *R* genes conferring disease resistance to bacteria, fungi, viruses, nematodes, and insects has identified several structural features characteristic to plant *R* genes. The majority are members of tightly linked multigene families and all *R* genes characterised so far, with the exception of Pto, encode leucine-rich repeats (LRRs), structures shown to be involved in protein-protein interactions. LRR containing *R* genes can be divided into two classes based on the presence of a putative tripartite nucleotide-binding site (NBS). *R* genes of the NBS-LRR class comprise motifs that are shared with animal apoptosis regulatory proteins. The second class of LRR containing *R* genes encompasses genes with a predicted hydrophobic membrane-anchoring domain with a predicted extracellular N-terminal LRR motif. The recently cloned resistance gene *R1* conferring race specific resistance to late blight belongs to the NBS-LRR class of *R* genes.

[0006] The invention provides an isolated or recombinant nucleic acid essentially corresponding to a cluster of genes identifiable by phylogenetic tree analyses, preferably of the encoded amino acid sequence, for example when comparing functionalities, as corresponding to the *Rpi-blb, RGC1-blb, RGC3-blb and RGC4-blb* gene cluster (herein also called the *Rpi-blb* gene cluster) of figure 9.

[0007] Phylogenetic tree analysis is carried out as follows. First a multiple sequence alignment is made of the nucleic acid sequences an/or preferably of the deduced amino acid sequences of the genes to be analysed using CLUSTALW (http://www2.ebi.ac.uk/clustalw), which is in standard use in the art. ClustalW produces a .dnd file, which can be read by TREEVIEW (http://taxonomy.zoology.gla.ac.uk/rod/rod.html). The phylogenetic tree depicted in Figure 9 is a phylogram.

[0008] Phylogenetic studies of the deduced amino acid sequences of *Rpi-blb, RGC1-blb, RGC3-blb, RGC4-blb* and those of the most similar genes from the art (as defined by the BLASTX) derived from diverse species, using the

Neighbour-Joining method of Saitou and Nei (1987), shows that corresponding genes or functional fragments thereof of the *Rpi-blb* gene cluster can be placed in a separate branch (Figure 9).

[0009] Sequence comparisons between the four members of the *Rpi-blb* gene cluster identified on 8005-8 BAC clone SPB4 show that sequence homology within the *Rpi-blb* gene cluster varies between 70% and 81% at the amino acid sequence level, providing a convenient rule of thumb: a nucleic acid encoding a peptide of at least 15 amino acids, preferably of at least 25 amino acids, most preferably of at least 50 amino acids, having at least 70% homology to corresponding stretches of peptides selected from any of the proteins encoded by the *Rpi-blb, RGC1-blb, RGC3-blb* and *RGC4-blb* genes are provided as functional fragment, herewith. The deduced amino acid sequence of *Rpi-blb* shares the highest overall homology with *RGC3-blb* (81% amino-acid sequence identity; Table 4). When the different domains are compared it is clear that the effector domains present in the N-terminal halves of the proteins (coiled-coil and NBS-ARC domains) share a higher degree of homology (91% sequence identity) than the C-terminal halves of these proteins which are thought to contain the recognition domains (LRRs; 71% amino acid sequence identity). Comparison of all four amino-acid sequences revealed a total of 104 Rpi-blb specific amino acid residues (Figure 10). The majority of these are located in the LRR region (80/104). Within the latter region, these specific residues are concentrated in the LRR subdomain xxLxLxxxx. The relative frequency of these specific amino-acid residues within this LRR subdomain is more than two times higher (28.3%) than that observed in the rest of the LRR domain (12.3%). The residues positioned around the two conserved leucine residues in the consensus xxLxxLxxxx are thought to be solvent exposed and are therefore likely to be involved in creating/maintaining recognition specificity of the resistance protein.

[0010] Sequences of additional members of the *Rpi-blb* gene cluster can be obtained by screening genomic DNA or insert libraries, e.g. BAC libraries with primers based on signature sequences of the *Rpi-blb* gene. Screening of various *Solanum* BAC libraries with primer sets A and/or B (Table 2 and Figure 7) identified numerous *Rpi-blb* homologues derived from different *Solanum* species. Alignment of these additional sequences with those presented in Figure 10 will help identify additional members of the *Rpi-blb* gene cluster and specific amino acid residues therein responsible for *P. infestans* resistance specificity. Furthermore, testing additional sequences in the above described phylogenetic tree analyses, e.g. using the Neighbour-Joining method of Saitou and Nei (1987), provides additional identification of genes belonging to the *Rpi-blb* gene cluster.

[0011] The invention provides the development of an intraspecific mapping population of *S. bulbocastanum* that segregated for race non-specific resistance to late blight. The resistance was mapped on chromosome 8, in a region located 0.3 cM distal from CT88. Due to the race non-specific nature of the resistance, *S. bulbocastanum* late blight resistance has always been thought to be *R* gene independent. However, with the current invention we demonstrate for the first time that *S. bulbocastanum* race non-specific resistance is in fact conferred by a gene bearing similarity to an *R* gene of the NBS-LRR type.

[0012] The invention further provides the molecular analysis of this genomic region and the isolation by map based cloning of a DNA-fragment of the resistant parent that harbours an *R* gene, designated *Rpi-blb*. This DNA-fragment was subcloned from an approximately 80 kb bacterial artificial chromosome (BAC) clone which contained four complete *R* gene-like sequences in a cluster-like arrangement. Transformation of a susceptible potato cultivar by *Agrobacterium tumefaciens* revealed that one of the four *R* gene-like sequences corresponds to *Rpi-blb* that provides the race non-specific resistance to late blight. Characterisation of the *Rpi-blb* gene showed that it is a member of the NBS-LRR class of plant *R* genes. The closest functionally characterised sequences of the prior art are members of the *I2* resistance gene family in tomato. These sequences have an overall amino acid sequence identity of approximately 32% with that of *Rpi-blb*.

[0013] Thus, in a first embodiment, the invention provides an isolated or recombinant nucleic acid, said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* family with race non-specific resistance against an oomycete pathogen.

[0014] Isolation of the gene as provided here that codes for the desired resistance trait against late blight and subsequent transformation of existing potato and tomato cultivars with this gene now provides a much more straightforward and quicker approach when compared to introgression breeding. The results provided here offer possibilities to further study the molecular basis of the plant pathogen interaction, the ecological role of *R* genes in a wild Mexican potato species and are useful for development of resistant potato or tomato cultivars by means of genetic modification.

[0015] In contrast to the *R* genes cloned and described so far, the gene we provide here is the first isolated *R* gene from a *Solanum* species that provides race non-specific resistance against an oomycete pathogen. Notably, the invention provides here a nucleic acid wherein said *Solanum* species that is provided with the desired resistance comprises *S. tuberosum*. In particular, it is the first gene that has been isolated from a phylogenetically distinct relative of cultivated potato, *S. bulbocastanum*, for which it was shown by complementation assays, that it is functional in *S. tuberosum*. These data imply that the gene *Rpi-blb* can easily be applied in potato production without a need for time-consuming and complex introgression breeding.

[0016] The following definitions are provided for terms used in the description and examples that follow.

- *Nucleic acid:* a double or single stranded DNA or RNA molecule.
- *Oligonucleotide:* a short single-stranded nucleic acid molecule.
- *Primer:* the term primer refers to an oligonucleotide that can prime the synthesis of nucleic acid.
- *Homology:* homology may be defined and determined by the TBLASTN or TBLASTP program for nucleic acid or amino acid sequences, respectively, of Altschul *et al.* (1990), which is in standard use in the art, or, and this may be preferred, the standard program BestFit, which is part of the Wisconsin Package, Version 8, September 1994, (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA, Wisconsin 53711). Homology may be at the nucleotide sequence and/or encoded amino acid sequence level. Preferably the nucleic acid and/or amino acid sequence shares at least 50%, or 60% homology, most preferably at least about 70%, or 80% or 90% homology with the sequence as depicted in Fig. 6. As shown in Table 4, the closest functionally characterised sequence of the prior art (members of the *I2 Fusarium* resistance gene cluster in tomato) has a much lower level of amino acid sequence identity than this (32% with respect to that of *Rpi-blb*). Homology within the *R* gene cluster of the present invention varies between 70% and 81% at the amino acid sequence level. Alternatively, a sequence is defined as belonging to the same cluster when numerous sequences are compared according to the Neighbour-Joining method of Saitou and Nei (1987).
- *Promoter:* the term "promoter" is intended to mean a short DNA sequence to which RNA polymerase and/or other transcription initiation factors bind prior to transcription of the DNA to which the promoter is functionally connected, allowing transcription to take place. The promoter is usually situated upstream (5') of the coding sequence. In its broader scope, the term "promoter" includes the RNA polymerase binding site as well as regulatory sequence elements located within several hundreds of base pairs, occasionally even further away, from the transcription start site. Such regulatory sequences are, e.g., sequences that are involved in the binding of protein factors that control the effectiveness of transcription initiation in response to physiological conditions. The promoter region should be functional in the host cell and preferably corresponds to the natural promoter region of the *Rpi-blb* resistance gene. However, any heterologous promoter region can be used as long as it is functional in the host cell where expression is desired. The heterologous promoter can be either constitutive or regulatable. A constitutive promoter such as the CaMV 35S promoter or T-DNA promoters, all well known to those skilled in the art, is a promoter which is subjected to substantially no regulation such as induction or repression, but which allows for a steady and substantially unchanged transcription of the DNA sequence to which it is functionally bound in all active cells of the organism provided that other requirements for the transcription to take place is fulfilled. A regulatable promoter is a promoter of which the function is regulated by one or more factors. These factors may either be such which by their presence ensure expression of the relevant DNA sequence or may, alternatively, be such which suppress the expression of the DNA sequence so that their absence causes the DNA sequence to be expressed. Thus, the promoter and optionally its associated regulatory sequence may be activated by the presence or absence of one or more factors to affect transcription of the DNA sequences of the genetic construct of the invention. Suitable promoter sequences and means for obtaining an increased transcription and expression are known to those skilled in the art.
- *Terminator*: the transcription terminator serves to terminate the transcription of the DNA into RNA and is preferably selected from the group consisting of plant transcription terminator sequences, bacterial transcription terminator sequences and plant virus terminator sequences known to those skilled in the art.
- *Gene:* the term "gene" is used to indicate a DNA sequence which is involved in producing a polypeptide chain and which includes regions preceding and following the coding region (5'-upstream and 3'-downstream sequences) as well as intervening sequences, the so-called introns, which are placed between individual coding segments (so-called exons) or in the 5'-upstream or 3'-downstream region. The 5'-upstream region may comprise a regulatory sequence that controls the expression of the gene, typically a promoter. The 3'-downstream region may comprise sequences which are involved in termination of transcription of the gene and optionally sequences responsible for polyadenylation of the transcript and the 3' untranslated region. The term "resistance gene" is an isolated nucleic acid according to the invention said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* family with resistance against an oomycete pathogen, said nucleic acid preferably comprising a sequence as depicted in Fig. 6 or part thereof, or a homologous sequence with essentially similar functional and structural characteristics. A functionally equivalent fragment of such a resistance gene or nucleic acid as provided by the invention encodes a fragment of a polypeptide having an amino acid sequence as depicted in Fig. 8 or part thereof, or a homologous and/or functionally equivalent polypeptide, said fragment exhibiting the characteristic of providing at least partial resistance to an oomycete infection such as caused by *P. infestans* when incorporated and expressed in a plant or plant cell.
- *Resistance gene product:* a polypeptide having an amino acid sequence as depicted in Fig. 8 or part thereof, or a homologous and/or functionally equivalent polypeptide exhibiting the characteristic of providing at least partial resistance to an oomycete infection such as caused by *P. infestans* when incorporated and expressed in a plant or plant cell.

- R_0 plant: primary regenerant from a transformation experiment, also denoted as transformed plant or transgenic plant.

[0017] In the present invention we have identified and isolated the resistance gene *Rpi-blb*, which confers race non-specific resistance to *Phytophthora infestans*. The gene was cloned from a *Solanum bulbocastanum* genotype that is resistant to *P. infestans*. The isolated resistance gene according to the invention can be transferred to a susceptible host plant using *Agrobacterium* mediated transformation or any other known transformation method, and is involved in conferring the host plant resistant to plant pathogens, especially *P. infestans*. The host plant can be potato, tomato or any other plant, in particular a member of the *Solanacae* family that may be infected by such a plant pathogen. The present invention provides also a nucleic acid sequence comprising the *Rpi-blb* gene, or a functionally equivalent fragment thereof, which is depicted in Figure 6.

[0018] With the *Rpi-blb* resistance gene or functionally equivalent fragment thereof according to the invention, one has an effective means of control against plant pathogens, since the gene can be used for transforming susceptible plant genotypes thereby producing genetically transformed plants having a reduced susceptibility or being preferably resistant to a plant pathogen. In particular, a plant genetically transformed with the *Rpiblb* resistance gene according to the invention has a reduced susceptibility to *P. infestans*.

[0019] In a preferred embodiment the *Rpi-blb* resistance gene comprises the coding sequence provided in Figure 6B or any corresponding or homologous sequence preceded by a promoter region and/or followed by a terminator region. The promoter region should be functional in plant cells, and preferably correspond to the native promoter region of the *Rpi-blb* gene. However, a heterologous promoter region that is functional in plant cells can be used in conjunction with the coding sequences.

[0020] In addition the invention relates to the *Rpi-blb* resistance gene product which is encoded by the *Rpi-blb* gene according to the invention and which has an amino acid sequence provided in Figure 8, or which is homologous to the deduced amino acid sequence or part thereof.

[0021] The invention also provides a vector comprising a nucleic acid as provided herein, said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* family with resistance against an oomycete pathogen, or a functionally equivalent isolated or recombinant nucleic acid in particular wherein said member comprises *S. tuberosum* or *Lycopersicon esculentum*.

[0022] The invention also provides a host cell comprising a nucleic acid or a vector according to the invention. An example of said host cell is provided in the detailed description herein. In a particular embodiment, said host cell comprises a plant cell. As a plant cell a cell derived from a member of the *Solanacae* family is preferred, in particular wherein said member comprises *S. tuberosum* or *Lycopersicon esculentum*. From such a cell, or protoplast, a transgenic plant, such as transgenic potato plant or tomato plant with resistance against an oomycete infection can arise. The invention thus also provides a plant, or tuber root, fruit or seed or part or progeny derived thereof comprising a cell according to the invention.

[0023] Furthermore, the invention provides a proteinaceous substance, exhibiting the characteristic of providing at least partial resistance to an oomycete infection such as caused by *P. infestans* when incorporated and expressed in a plant or plant cell. In particular such a proteinaceous substance is provided that is encoded by a nucleic acid according to the invention. In a preferred embodiment, the invention provides a proteinaceous substance comprising an amino acid sequence as depicted in figure 8 or part thereof. Such a proteinaceous substance is for example useful for obtaining a binding molecule directed at said substance. Particular easy to obtain, merely by immunizing an appropriate animal and harvesting a polyclonal serum or a monoclonal antibody, are antibodies or fragments thereof, but other binding molecules such as synthetic antibodies or peptide mimics thereof can for example be obtained by phage display methods.

[0024] Furthermore, the invention provides a binding molecule directed at a nucleic acid according to the invention. For example, the *Rpi-blb* gene can be used for the design of oligonucleotides complementary to one strand of the DNA sequence as depicted in Figure 7 and Table 2. Such oligonucleotides as provided herein are useful as probes for library screening, hybridisation probes for Southern/Northern analysis, primers for PCR, for use in a diagnostic kit for the detection of disease resistance and so on. Such oligonucleotides are useful fragments of an isolated or recombinant nucleic acid as provided herein, said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* family with resistance against an oomycete fungus, or a functionally equivalent isolated or recombinant nucleic acid, in particular wherein said member comprises *S. tuberosum* or *Lycopersicon esculentum*. They can be easily selected from a sequence as depicted in figure 6 or part thereof. A particular point of recognition comprises the LRR domain as identified herein. Such a binding molecule according to the invention is used as a probe or primer, for example provided with a label, in particular wherein said label comprises an excitable moiety which makes it useful to detect the presence of said binding molecule.

[0025] The invention furthermore provides a method for selecting a plant or plant material or progeny thereof for its susceptibility or resistance to an oomycete infection comprising testing at least part of said plant or plant material or

progeny thereof for the presence or absence of a nucleic acid, said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* family with resistance against an oomycete fungus, or for the presence of said gene product, said method preferably comprising contacting at least part of said plant or plant material or progeny thereof with a binding molecule according the invention and determining the binding of said molecule to said part. Said method is particularly useful wherein said oomycete comprises *P. infestans*, allowing to select plants or planting material for resistance against late blight, for example wherein said plant or material comprises *S. tuberosum*.

[0026] Also, the invention provides use of a nucleic acid or a vector or a cell or a substance or a binding molecule according to the invention in a method for providing a plant or its progeny with at least partial resistance against an oomycete infection, in particular wherein said oomycete comprises *P. infestans* especially wherein said plant comprises *S. tuberosum*, said method for providing a plant or its progeny with at least partial resistance against an oomycete infection comprising providing said plant or part thereof with a gene or functional fragment thereof comprising a nucleic acid, said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* family with resistance against an oomycete fungus, or providing said plant or part thereof with a nucleic acid or a vector or a cell or a substance according to the invention.

[0027] Furthermore, the invention provides an isolated *S. bulbocastanum*, or part thereof, such as a tuber or seed, susceptible to an oomycete infection caused by *P. infestans*.

[0028] The invention is further described in the detailed description below.

DESCRIPTION OF THE FIGURES

[0029]

Figure 1. Geographical map of Mexico indicating the origin of *Solanum bulbocastanum* accessions used to isolate the *Rpi-blb* gene. The letters a, b and c indicate the relative geographical origins of the used *S. bulbocastanum* accessions.

Figure 2. Genetic linkage maps of the *Rpi-blb* locus on chromosome 8 of *S. bulbocastanum.* Horizontal lines indicate the relative positions of markers linked to late blight resistance. Distances between markers are indicated in centimorgans. **A.** Genetic position of the *Rpi-blb* locus relative to markers TG513, CT88 and CT64 (n=508 genotypes). **B.** High density genetic linkage map of the *Rpi-blb* locus (n=2109 genotypes).

Figure 3. Physical map of the *Rpiblb* locus. **A.** Genetic and physical map of the *S. bulbocastanum* genomic region containing Rpi_{blb1}. Vertical arrows indicate the relative positions of markers linked to resistance. Numbers above the horizontal line indicate the number of recombinants identified between the flanking markers in 2109 progeny plants. Rectangles represent bacterial artificial chromosome (BAC) clones. **B.** Relative positions of candidate genes for late blight resistance on BAC SPB4. **C.** Schematic representation of the *Rpi-blb* gene structure. Horizontal lines indicate exons. Open boxes represent coding sequence. Lines angled downwards indicate the position of a 678-nucleotide long intron sequence.

Figure 4. Southern blot analysis of the BAC contig spanning the *Rpi-blb* locus. Names above each lane represent the names of BAC clones. The names of the restriction enzymes used to digest the BAC DNA prior to Southern blotting are indicated.

Figure 5. Detached leaf disease assays. **A.** Resistant (left), intermediate (centre) and susceptible (right) phenotypes found in the *S. bulbocastanum* mapping population B8 6 days post inoculation (d.p.i) with *P. infestans* sporangiospore droplets. **B.** Genetic complementation for late blight resistance. Characteristic disease phenotypes of leaves derived from transgenic potato plants harbouring *RGC1-blb, RGC2-blb, -blb* or *RGC4-blb* 6 d.p.i. with *P. infestans* sporangiospore droplets. Genetic constructs harbouring the RGCs were transferred to the susceptible potato cultivar Impala through *Agrobacterium* mediated transformation.

Figure 6. Nucleic acid sequences of the *Rpi-blb* gene cluster members. **A.** Coding nucleic acid sequence of the *Rpi-blb* gene. **B.** Coding nucleic acid sequence of the *Rpi-blb* gene including the intron sequence (position 428-1106). **C.** Sequence of the 7.35 kb *Sau*3AI genomic DNA fragment of *S. bulbocastanum* BAC SPB4 present in pRGC2-blb, the genetic construct used for genetic complementation for late blight resistance. The genetic construct harbours the *Rpi-blb* gene. The initiation codon (ATG position 2648-2650) and the termination codon (TAA position 6237-6239) are underlined. **D.** Coding nucleic acid sequence of *RGC1-blb* including the intron sequence (position 428-708). **E.** Coding nucleic acid sequence of *RGC3-blb* including the intron sequence (position 428-1458). **F.** Coding nucleic acid sequence of *RGC4-blb* including intron sequences (positions 434-510, 543-618

and 743-1365).

Figure 7. Relative primer positions. The horizontal bar represents the coding sequence of the *Rpi-blb* gene. Numbers represent nucleotide positions. Horizontal arrows indicate relative primer positions and orientations. GSP1 and GSP2 represent nested gene specific primers used for 3' RACE experiments. GSP3 and GSP4 represent nested gene specific primers used for 5' RACE experiments. A(F), A(R), B(F) and B(R) are primers used to amplify *Rpi-blb* homologues. The position of the restriction site *NsiI* used to make domain swaps between *Rpi-blb* homologues is indicated.

Figure 8. Deduced Rpi-blb protein sequence. The amino acid sequence deduced from the DNA sequence of *Rpi-blb* is divided into three domains (A-C), as described in the text. Hydrophobic residues in domain A that form the first and fourth residues of heptad repeats of potential coiled-coil domains are underlined. Conserved motifs in R proteins are written in lowercase and in italic in domain B. Residues matching the consensus of the cytoplasmic LRR are indicated in bold in domain C. Dots in the sequence have been introduced to align the sequence to the consensus LRR sequence of cytoplasmic LRRs.

Figure 9. Phylogenetic tree of state of the art sequences which share some homology to the deduced amino acid sequence of *Rpi-blb* and its gene cluster members *RGC1-blb, RGC3-blb* and *RGC4-blb*. The tree was made according to the Neighbour-Joining method of Saitou and Nei (1987). An asterix indicates that the gene has been assigned a function. The *Rpi-blb* gene cluster is boxed.

Figure 10. Alignment of the deduced protein products encoded by *Rpi-blb, RGC1-blb, RGC3-blb* and *RGC4-blb.* The complete amino acid sequence of Rpi-blb is shown and amino acid residues from RGC1-blb, RGC3-blb and RGC4-blb that differ from the corresponding residue in Rpi-blb. Dashes indicate gaps inserted to maintain optimal alignment. Amino acid residues that are specific for Rpi-blb, when compared to those at corresponding positions in RGC1-blb, RGC3-blb and RGC4-blb, are highlighted in bold. The regions of the LRRs that correspond to the consensus L..L..L.L..C/N/S..a..aP are underlined. Conserved motifs in the NBS domain are indicated in lowercase.

Figure 11. Schematic overview of domain swaps made between *Rpi-blb* and homologues *RGC1-blb* and *RGC3-blb.* The vertical dotted line indicates the position of the *NsiI* site used to make the swaps. R and S indicate whether transgenic plants harbouring specific chimeric constructs are resistant or susceptible to late blight infection, respectively.

Detailed description

[0030] For the mapping of the *Rpi-blb* resistance gene an intraspecific mapping population of *S. bulbocastanum* was developed. A crucial step in this process was the identification of susceptible *S. bulbocastanum* genotypes. For this purpose several *S. bulbocastanum* accessions originating from different clusters/areas in Mexico were analysed for *P. infestans* resistance or susceptibility in a detached leaf assay (Table 1 and Figure 1). The screened accessions BGRC 8008 and BGRC 7999 contained no susceptible genotypes. However in the accessions BGRC 8005, BGRC 8006 and BGRC 7997, susceptibility was found in 9%, 7% and 14 % of the analysed seedlings, respectively. A *P. infestans* susceptible clone of accession BGRC 8006 was subsequently selected and crossed with a resistant clone of accession BGRC 8005. The resulting F1 population was used to map the *Rpi-blb* locus and is hereafter referred to as the B8 population.

[0031] Initial screening of 42 B8 genotypes for resistance to *P. infestans* in a detached leaf assay suggested that *P. infestans* resistance in *S. bulbocastanum* accession 8005 could be caused by a single dominant *R* gene, or a tightly linked gene cluster. Of the 42 genotypes tested, 22 scored resistant and 16 susceptible in a repeated experiment. Resistance phenotypes of the remaining 4 seedlings remained unclear. In order to determine the chromosome position of this *S. bulbocastanum* resistance, B8 genotypes with an undoubted phenotype were used for marker analysis. The chromosome 8 specific marker TG330 (Table 2) was found to be linked in repulsion phase with the resistant phenotype, as only one recombinant was obtained between this marker and resistance in 12 B8 genotypes. Furthermore, chromosome 8 marker CT88 (Table 2) was found to be completely linked in repulsion phase to resistance, indicating that the locus responsible for resistance, designated *Rpi-blb*, was located in this region of chromosome 8. For this reason, tomato chromosome 8 specific markers that map proximal and distal to CT88 (TG513 and CT64; Tanksley et al., 1992; Table 2) were developed into CAPS markers and tested in 512 B8 genotypes with known resistance phenotypes. A total of five CT64-CT88 recombinant genotypes and 41 CT88-TG513 recombinant genotypes were identified in this screen (Figure 2A). The resistance locus *Rpi-blb* was mapped 1 recombination event distal to marker CT88 (Figure 2A).

[0032] Fine mapping of the *Rpi-blb* locus was carried out with CAPS markers derived from left (L) and right (R) border

sequences of BAC clones isolated from a BAC library prepared from the resistant *S. bulbocastanum* genotype BGRC 8005-8. The BAC library was initially screened with markers CT88 and CT64. BAC clones identified with these markers were used as seed BACs for a subsequent chromosome walk to the *Rpi-blb* locus. A total of 2109 B8 genotypes were screened for recombination between markers TG513 en CT64. All recombinant genotypes (219/2109) were subsequently screened with all available markers in the CT88-CT64 genetic interval. These data together with the disease resistance data of each recombinant, obtained through detached leaf assays, positioned the *Rpi-blb* locus between markers SPB33L and B149R, a 0.1 cM genetic interval (4/2109 recombinants) physically spanned by the overlapping BAC clones SPB4 and B49 (Figures 2b and 3). Within this interval resistance cosegregated with the BAC end marker SPB42L, the sequence of which shared homology to the *Fusarium I2* gene cluster from tomato (Ori et al., 1997, Simons et al., 1998). Southern analyses of BAC clones spanning the SP33L-B149R interval using a ^{32}P-labeled PCR fragment of marker SPB42L as a probe revealed the presence of at least 4 copies of this *R* gene like sequence within the *Rpi-blb* interval (Figure 4). Moreover, all of these copies were present on BAC SPB4. Sequencing and annotation of the complete insert of this BAC clone indeed identified four complete *R* gene candidates (*RGC1-blb, RGC2-blb, RGC3-blb and RGC4-blb*) of the NBS-LRR class of plant *R* genes. A PCR-marker that was located in-between *RGC1-blb* and *RGC4-blb* revealed recombination between *P. infestans* resistance and *RGC4-blb,* ruling out the possibility of *RGC4-blb* being *Rpi-blb*. Despite this finding, all four RGCs were selected for complementation analysis.

[0033] Genomic fragments of approximately 10 kb harbouring *RGC1-blb, RGC2-blb, RGC3-blb* or *RGC4-blb* were subcloned from BAC SPB4 into the binary plant transformation vector pBINPLUS (van Engelen et al., 1995) and transferred to a susceptible potato cultivar using standard transformation methods. Primary transformants were tested for *P. infestans* resistance as described in Example 1. Only the genetic construct harbouring *RGC2-blb* was able to complement the susceptible phenotype; 86% of the R_0(*RGC2-blb*) plants were resistant (Table 3) whereas all *RGC1-blb, RGC3-blb* and *RGC4-blb* containing primary transformants were completely susceptible to *P. infestans*. The resistant *RGC2-blb* containingtransformants showed similar resistance phenotypes as the *S. bulbocastanum* resistant parent (Figure 5). *RGC2-blb* was therefore designated the *Rpi-blb* gene, the DNA sequence of which is provided in Figure 6.

EXAMPLE 1: DISEASE ASSAY

[0034] The phenotype of *S. bulbocastanum* and transgenic *S. tuberosum* genotypes for resistance to *P. infestans* was determined by detached leaf assays. Leaves from plants grown for 6 to 12 weeks in the greenhouse were placed in pieces of water-saturated florists foam, approximately 35x4x4 cm, and put in a tray (40 cm width, 60 cm length and 6 cm height) with a perforated bottom. Each leaf was inoculated with two droplets or more (25 µl each) of sporangiospore solution on the abaxial side. Subsequently, the tray was placed in a plastic bag on top of a tray, in which a water-saturated filter paper was placed, and incubated in a climate room at 17°C and a 16h/8h day/night photoperiod with fluorescent light (Philips TLD50W/84HF). After 6 days, the leaves were evaluated for the development of *P. infestans* disease symptoms. Plants with leaves that clearly showed sporulating lesions 6 days after inoculation were considered to have a susceptible phenotype whereas plants with leaves showing no visible symptoms or necrosis at the side of inoculation in the absence of clear sporulation were considered to be resistant. The assay was performed with *P. infestans* complex isolate 655-2A, which was obtained from Plant Research International BV (Wageningen, The Netherlands).

EXAMPLE 2: MAPPING OF THE *Rpi-blb* RESISTANCE LOCUS

Plant material

[0035] In order to produce an intraspecific mapping population that segregated for the *P. infestans* resistance gene present in *S. bulbocastanum* accession BGRC 8005 (CGN 17692, PI 275193), a susceptible *S. bulbocastanum* genotype was required. Several *S. bulbocastanum* accessions originating from different clusters/areas in Mexico were analysed for *P. infestans* resistance or susceptibility in a detached leaf assay (Table 1 and Figure 1). In accession BGRC 8008 and BGRC 7999 no susceptibility was detected. In accession BGRC 8005, BGRC 8006 and BGRC 7997 susceptibility was only present in 9%, 7% and 14 % of the analysed seedlings, respectively. Thus, only a few susceptible *S. bulbocastanum* genotypes were obtained.

[0036] The intraspecific mapping population of S. *bulbocastanum* (B8) was produced by crossing a *P. infestans* susceptible clone of accession BGRC 8006 with a resistant clone of accession BGRC 8005. DNA of 2109 progeny plants was extracted from young leaves according to Doyle and Doyle (1989).

CAPS marker analysis

[0037] For PCR analysis, 15 µl reaction mixtures were prepared containing 0.5 µg DNA, 15 ng of each primer, 0.2

mM of each dNTP, 0.6 units Taq-polymerase (15 U/µl, SphaeroQ, Leiden, The Netherlands), 10 mM Tris-HCl pH 9, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100 and 0.01% (w/v) gelatin. The PCRs were performed using the following cycle profile: 25 seconds DNA denaturation at 94°C, 30 seconds annealing (see Table 1) and 40 seconds elongation at 72°C. As a first step in PCR-amplification DNA was denatured for 5 min at 94°C and finalised by an extra 5 min elongation step at 72°C. The amplification reactions were performed in a Biometra® T-Gradient or Biometra® Uno-II thermocycler (Westburg, Leusden, The Netherlands). Depending on the marker, the PCR product was digested with an appropriate restriction enzyme. An overview of the markers including primer sequences, annealing temperature and restriction enzymes, is given in Table 2. Subsequently, the (digested) PCR products were analysed by electrophoresis in agarose or acrylamide gels. For acrylamide gel analysis, the CleanGel DNA Analysis Kit and DNA Silver Staining Kit (Amersham Pharmacia Biotech Benelux, Roosendaal, the Netherlands) were used.

Genetic mapping of the *Rpi-blb* locus

[0038] Initially a small group of 42 progeny plants of the B8 population was screened for resistance to *P. infestans* in a detached leaf assay. Plants with leaves that clearly showed sporulating lesions 6 days after inoculation were considered to have a susceptible phenotype whereas plants with leaves showing no visible symptoms or necrosis at the side of inoculation in the absence of clear sporulation were considered to be resistant. Of the 42 seedlings, 22 scored resistant and 16 susceptible. The phenotype of the remaining 4 seedlings remained unclear in this initial phase. These data indicated that resistance could be due to a single dominant gene or a tightly linked gene cluster. In order to determine the chromosome position, seedlings with a reliable phenotype were used for marker analysis. Chromosome 8 marker TG330 was found to be linked in repulsion with the resistant phenotype, as only one recombinant was obtained between this marker and resistance in 12 B8 seedlings. Furthermore, chromosome 8 marker CT88 was found to be completely linked in repulsion phase to resistance, indicating that a resistance gene was located on chromosome 8.

[0039] Subsequently, chromosome 8 specific markers that had been mapped proximal and distal to CT88 (Tanksley et al., 1992) were developed to CAPS markers. In order to map these markers more precisely, another 512 individuals of the B8 population were screened for late blight resistance using the detached leaf disease assay. Simultaneously, plants were scored for the markers CT64, CT88 and TG513. For 5 seedlings, recombination was detected between markers CT64 and CT88, while 41 seedlings were recombinant between markers CT88 and TG513 (Figure 2A). The resistance gene *Rpi-blb* was mapped in between markers CT64 and CT88. In this stage, the positioning of CT88 proximal to *Rpi-blb* was based on only one recombined seedling.

[0040] In order to determine the position of *Rpi-blb* more precisely relative to the available markers, another 1555 seedlings of the B8 population were grown and analysed for recombination between the markers TG513 and CT64. Thus, a total of 2109 individual offspring clones of the B8 population were screened. Recombination between markers TG513 en CT64 was detected in 219 of these seedlings (10.4 cM). All of the recombinants were screened with marker CT88 and phenotyped for the resistance trait by making use of the detached leaf assay. In agreement with earlier results, the *Rpi-blb* gene was mapped in between markers CT88 and CT64 (Figure 2B).

EXAMPLE 3: CONSTRUCTION OF A *S. BULBOCASTANUM* BAC LIBRARY AND CONSTRUCTION OF A CONTIGUOUS BAC CONTIG SPANNING THE *Rpi-blb* LOCUS

BAC library construction

[0041] A resistant clone of *S. bulbocastanum* (blb) accession BGRC 8005 (CGN 17692, PI 275193) heterozygous for the *Rpi-blb* locus, was used as source DNA for the construction of a genomic BAC library, hereafter referred to as the 8005-8 BAC library. High molecular weight DNA preparation and BAC library construction were carried out as described in Rouppe van der Voort et al. (1999). Approximately 130.000 clones with an average insert size of 100 kb, which corresponds to 15 genome equivalents were finally obtained. A total of approximately 83.000 individual clones were stored in 216 384-well microtiter plates (Invitrogen, The Netherlands) containing LB freezing buffer (36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM citrate, 0.4 mM $MgSO_4$, 6.8 mM $(NH_4)_2SO_4$, 4.4 % V/V glycerol, 12.5 µg/ml chloramphenicol in LB medium) at -80°C. Another 50.000 clones were stored as bacterial pools containing ~1000 white colonies. These were generated by scraping the colonies from the agar plates into LB medium containing 18% glycerol and 12.5 µg/ml chloramphenicol using a sterile glass spreader. These so-called super pools were also stored at -80°C.

Screening of the BAC library and construction of a physical map of the *Rpi-blb* locus

[0042] The 8005-8 BAC library was initially screened with CAPS markers CT88 and CT64. This was carried out as

follows. For the first part of the library of approximately 83.000 clones stored in 384 well microtiter plates, plasmid DNA was isolated using the standard alkaline lysis protocol (Sambrook *et al.*, 1989) from pooled bacteria of each plate to produce 216 plate pools. To identify individual BAC clones carrying the CAPS markers the plate pools were screened by PCR. Once an individual plate pool was identified as being positive for a particular CAPS marker the positive row and positive column were identified through a two dimensional PCR screening. For this purpose, the mother 384-well plate was replicated twice on LB medium containing chloramphenicol (12.5 µg/ml). After growing the colonies for 16 h at 37°C one plate was used to scrape the 24 colonies of each row together and the other plate was used to scrape the 16 colonies of each column together. Bacteria of each row or column were resuspended in 200 µl TE buffer. CAPS marker analysis on 5 µl of these bacterial suspensions was subsequently carried out leading to the identification of single positive BAC clones. For the second part of the library, stored as 50 pools of approximately 1000 clones, plasmid DNA was isolated from each pool of clones using the standard alkaline lysis protocol and PCR was carried out to identify positive pools. Bacteria corresponding to positive pools were diluted and plated on LB agar plates containing chloramphenicol (12.5 µg/ml). Individual white colonies were subsequently picked into 384-well microtiter plates and single positive BAC clones subsequently identified as described above. Names of BAC clones isolated from the super pools carry the prefix SP (e.g. SPB33).

[0043] Insert sizes of BAC clones were estimated as follows. Positive BAC clones were analysed by isolating plasmid DNA from 2 ml overnight cultures (LB medium supplemented with 12.5 mg/ml chloramphenicol) using the standard alkaline lysis miniprep protocol and resuspended in 20 µl TE. Plasmid DNA (10 µl) was digested with 5 U *Not*I for 3 h at 37°C to free the genomic DNA from the pBeloBAC11 vector. The digested DNA was separated by CHEF electrophoresis in a 1% agarose gel in 0.5 X TBE at 4°C using a BIORAD CHEF DR II system (Bio-Rad Laboratories, USA) at 150 volts with a constant pulse time of 14 sec for 16 h.

[0044] Screening of the 8005-8 BAC library with marker CT88 identified two positive BAC clones: B139 and B180, with potato DNA inserts of 130 and 120 kb, respectively (Figure 3A). Digestion of the CT88 PCR product generated from these BAC clones and several resistant and susceptible progeny plants of the B8 mapping population with *Mbo*I revealed that BAC139 carried the CT88 allele that was linked in *cis* to resistance. To identify the relative genome position of BAC B139, pairs of PCR primers were designed based on the sequence of the right (R) and left (L) ends of the insert. BAC end sequencing was carried out as described in Example 4 using 0.5 µg of BAC DNA as template. Polymorphic CAPS markers were developed by digesting the PCR products of the two parent genotypes of the B8 population and of two resistant and two susceptible progeny genotypes with several 4-base cutting restriction enzymes (Table 2). Screening of the 37 CT88-CT64 recombinant B8 genotypes mapped 5 of the 7 CT88-*Rpi-blb* recombinants between CT88 and B139R, indicating that marker B139R was relatively closer to the *Rpi-blb* locus than marker CT88. Screening of the 216 plate pools with B139R did not lead to the identification of a positive BAC clone. Screening of the 50 super pools identified the positive BAC clones SPB33 and SPB42 with DNA inserts of 85 and 75 kb, respectively (Figure 3A). Screening of the complete BAC library with SPB33L identified the positive BAC clones B149 and SPB4. BAC clone SPB4 contained the SPB33L allele that was linked *in cis* to resistance whereas BAC clone B149 did not. However, screening of the CT88-CT64 recombinant panel with B149R revealed that this BAC spanned the *Rpi-blb* locus. B149R was separated from the *Rpi-blb* locus by two recombination events (Figure 3A). Screening of the 8005-8 BAC library with B149R identified BAC clone B49 as having the B149R allele that was linked *in cis* to resistance. This BAC clone together with BAC clone SPB4 therefore formed a BAC contig that spanned the *Rpi-blb* locus (Figure 3).

EXAMPLE 4: SEQUENCE ANALYSIS OF BAC SPB4 AND IDENTIFICATION OF RESISTANCE GENE CANDIDATES WITHIN THE *Rpi-blb* LOCUS

[0045] Within the SPB33L-B149R interval resistance cosegregated with BAC end marker SPB42L, the sequence of which shared homology to NBS-LRR genes of the *Fusarium I2* gene cluster in tomato (Ori et al., 1997; Simons et al., 1998). Southern analyses of BAC clones spanning the SPB33L-B149R interval using a ^{32}P-labeled PCR fragment of marker SPB42L as a probe revealed the presence of at least 4 copies of this *R* gene like sequence within the *Rpi-blb* interval (Figure 4). Moreover, all of these copies were present on BAC SPB4. The DNA sequence of BAC clone SPB4 was therefore determined by shotgun sequence analysis. A set of random subclones with an average insert size of 1.5 kb was generated. 10 µg of CsCl purified DNA was sheared for 6 seconds on ice at 6 amplitude microns in 200 µl TE using an MSE soniprep 150 sonicator. After ethanol precipitation and resuspension in 20 µl TE the ends of the DNA fragments were repaired by T4 DNA polymerase incubation at 11°C for 25 minutes in a 50 µl reaction mixture comprising 1x T4 DNA polymerase buffer (New England BioLabs, USA), 1 mM DTT, 100 µM of all 4 dNTP's and 25 U T4 DNA polymerase (New England Biolabs, USA), followed by incubation at 65°C for 15 minutes. The sheared DNA was subsequently separated by electrophoresis on 1% SeaPlaque LMP agarose gel (FMC). The fraction with a size of 1.5-2.5 kb was excised from the gel and dialysed against 50 ml TE for 2 hr at 4°C. Dialysed agarose slices were then transferred to a 1.5 ml Eppendorf tube, melted at 70°C for 5 min, digested with 1 unit of GELASE (Epicentre Technologies, USA) per 100 mg of agarose gel for 1 hr at 45°C, and the DNA was subsequently precipitated. The 1.5-2.5 kb fragments

were ligated at 16°C in a *EcoRV* restricted and dephosphorylated pBluescript SK+ vector (Stratagene Inc.). The ligation mixture was subsequently used to transform ElectroMAX *E. coli* DH10B competent cells (Life Technologies, UK) by electroporation using the BioRad Gene Pulser. Settings on the BioRad Gene Pulser were as recommended for *E. coli* by the manufacturer. The cells were spread on Luria broth (LB) agar plates containing ampicillin (100 μg/ml), 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal) (64 μg/ml) and isopropyl-1-thio-β-D-galactoside (IPTG) (32 μg/ml). Plates were incubated at 37°C for 24 hours. Individual white colonies were grown in 96-well flat-bottom blocks (1.5 ml Terrific Broth medium containing 100 μg/ml ampicillin).

[0046] Plasmid DNA was isolated using the QIAprep 96 Turbo Miniprep system in conjunction with the BioRobot™ 9600 (QIAGEN) according to the manufacturers instructions. Sequencing reactions were performed using ABI PRISM BigDye™ Terminator cycle sequencing kit (Stratagene) according to the manufacturer's instructions. All clones were sequenced bi-directionally using universal primers. Sequence products were separated by capillary electrophoresis on a Perkin Elmer ABI 3700 DNA Analyzer.

[0047] The automated assembly of the shotgun reads was carried out using the Phred-Phrap programs (Ewing and Green, 1998; Ewing *et al.*, 1998). A total of 835 reads provided an overall BAC sequence coverage equal to 5x. Gaps between contigs were closed by primer walking or through a combinatorial PCR approach. The sequence was finally edited at Phred quality 40 (1 error every 10,000 nt) by manual inspection of the assembly using the Gap4 contig editor and re-sequencing of all low-quality regions. The complete sequence of the insert of BAC SPB4 consisted of 77,283 nucleotides.

[0048] Analysis of the contiguous sequence of BAC SPB4 using the computer programme GENSCAN (Burge and Karlin, 1997), GENEMARK (Lukashin and Borodovsky, 1998) and BLASTX (Altschul *et al.*, 1990) identified four complete *R* gene candidate sequences (*RGC1-blb, RGC2-blb, RGC3-blb and RGC4-blb*) belonging to the NBS-LRR class of plant *R* genes. A CAPS marker designed in between *RGC1-blb* and *RGC4-blb*, marker RGC1-4 revealed recombination between *P. infestans* resistance and *RGC4-blb,* ruling out the possibility of *RGC4-blb* being *Rpi-blb* (Figure 3A and B). Despite this finding, all four RGCs were selected for complementation analysis.

EXAMPLE 5: COMPLEMENTATION ANALYSIS

Subcloning of candidate genes and transformation to *Agrobacterium tumefaciens*

[0049] Genomic fragments of approximately 10 kb harbouring *RGC1-blb, RGC2-blb, RGC3-blb or RGC4-blb* were subcloned from BAC clone SPB4 into the binary plant transformation vector pBINPLUS (van Engelen et al., 1995). Restriction enzyme digestion of BAC clone SPB4 DNA and subsequent size selection was carried out as follows. Aliquots of ~1 μg DNA were digested with 1U, 0.1U or 0.01U of *Sau3A*I restriction enzym for 30 min. The partially digested BAC DNA was subjected to CHEF electrophoresis at 4°C in 0.5 X TBE using a linear increasing pulse time of 1-10 sec and a field strength of 6 V/cm for 16 hr. After electrophoresis, the agarose gel was stained with ethidium bromide to locate the region of the gel containing DNA fragments of approximately 10kb in size. This region was excised from the gel using a glass coverslip and dialysed against 50 ml TE for 2 hr at 4°C. Dialysed agarose slices were then transferred to a 1.5 ml Eppendorf tube, melted at 70°C for 5 min and digested with 1 unit of GELASE (Epicentre Technologies, USA) per 100 mg of agarose gel for 1 hr at 45°C. Ligation of the size selected DNA to *Bam*HI-digested and dephosphorylated pBINPLUS and subsequent transformation of ElectroMAX *E. coli* DH10B competent cells (Life Technologies, UK) with the ligated DNA was carried as described in Example 5, using the BioRad Gene Pulser for electroporation. The cells were spread on Luria broth (LB) agar plates containing kanamycin (50 μg/ml), Xgal (64 μg/ml) and IPTG (32 μg/ml). Plates were incubated at 37°C for 24 hours. Individual white colonies were grown in 96-well plates (100 μl LB medium containing 50 μg/ml kanamycin). A total of 480 clones were PCR screened for the presence of RGCs using primers SPB42LF and SPB42LR or RGC4F and RGC4R (Table 2.). Positive clones were selected for plasmid isolation and further characterisation. Identification of clones harbouring *RGC1-blb, RGC2-blb, RGC3-blb or RGC4-blb* was carried out by sequencing the SPB42L PCR fragments derived from positive clones. The relative position of the RGCs within a subclone was determined by sequencing the ends of the clone and subsequent comparison of the sequences to the complete BAC insert sequence. Finally four binary plasmids, pRGC1-blb, pRGC2-blb, pRGC3-blb and pRGC4-blb were selected and transferred to *Agrobacterium tumefaciens* strains AGL0 (Lazo *et al.*, 1991), LBA4404 (Hoekema *et al.*, 1983) or UIA143 (Farrand *et al.*, 1989) either by electroporation using the BioRad Gene Pulser or by conjugation. Settings on the BioRad Gene Pulser were as recommended for A. *tumefaciens* by the manufacturer. Conjugation was carried out as described by Simon *et al.* (1983). The cells were spread on Luria broth (LB) agar plates containing kanamycin (100 mg/l) and rifampicin (50 mg/l). Plates were incubated at 28°C for 48 hours. Small-scale cultures from selected colonies were grown in LB medium containing kanamycin (100 mg/l) and rifampicin (50 mg/l). Plasmid DNA was isolated as described previously and the integrity of the plasmids was verified by restriction analysis upon reisolation from *A. tumefaciens* and subsequent transformation to *E. coli*. *A tumefaciens* cultures harbouring a plasmid with the correct DNA pattern were used to transform a susceptible potato genotype.

Transformation of susceptible potato cultivar

[0050] *A. tumefaciens* strains were grown for 2 days at 28°C in 20 ml LB medium supplemented with 50 mg/l rifampicine and 25 mg/l kanamycin. Subsequently, 0.2 ml of *A. tumefaciens* culture was diluted in 10 ml LB medium containing the same antibiotics and grown overnight (28°C). The overnight culture was centrifuged (30 min, 2647 x g) and the pellet was resuspended in 50 ml MS medium (Murashige and Skoog, 1962) supplemented with 30 g/l sucrose (MS30).

[0051] Certified seed potatoes of cultivar Impala were peeled and surface sterilised for 30 min. in a 1% sodium hypochlorate solution containing 0.1 % Tween-20. Tubers were then washed thoroughly in large volumes of sterile distilled water (4 times, 10 min). Discs of approximately 2 mm thickness and 7 mm in diameter, were sliced from cylinders of tuber tissue prepared with a corkborer. The tuber discs were transferred into liquid MS30 medium containing *A. tumefaciens* and incubated for 15 min. After removing the *A. tumefaciens* solution, the tuber discs were transferred to regeneration medium containing MS30, 0.9 mg/l IAA, 3.6 mg/l zeatine riboside and 8 g/l agar (Hoekema et al., 1989). The plates were incubated at 24°C, 16 hour day-length (Philips TLD50W/84HF). After 48 hours of co-cultivation, the tuber discs were rinsed for 5 min in liquid MS medium including antibiotics, 200 mg/l vancomycin, 250 mg/l cefotaxim and 75 mg/l kanamycin, and transferred to regeneration medium supplemented with the same antibiotics. The plates were incubated at 24°C, 16 hour day-length (Philips TLD50W/84HF). Every three weeks, the tuber discs were transferred to fresh medium. Regenerating shoots were transferred to MS30 medium containing 75 mg/l kanamycin. Rooting shoots were propagated *in vitro* and tested for absence of *A. tumefaciens* cells by incubating a piece of stem in 3 ml LB medium (3 weeks, 37°C, 400 rpm). One plant of each transformed regenerant was transferred to the greenhouse.

Complementation of the susceptible phenotype in potato

[0052] Primary transformants were tested for *P. infestans* resistance as described in Example 1. Only the genetic construct harbouring *RGC2-blb* was able to complement the susceptible phenotype; 86% of the R_0*RGC2-blb* plants were resistant (Table 3) whereas all *RGC1-blb, RGC3-blb* and *RGC4-blb* containing primary transformants were completely susceptible to *P. infestans*. The resistant *RGC2-blb* transformants showed similar resistance phenotypes as the *S. bulbocastanum* resistant parent (Figure 5). *RGC2-blb* was therefore designated the *Rpi-blb* gene, the DNA sequence of which is provided in Figure 6.

Transformation of susceptible tomato

[0053] Seeds of the susceptible tomato line Moneymaker were rinsed in 70% ethanol to dissolve the seed coat and washed with sterile water. Subsequently, the seeds were surface-sterilised in 1.5% sodium hypochlorite for 15 minutes, rinsed three times in sterile water and placed in containers containing 140 ml MS medium pH 6.0 (Murashige and Skoog, 1962) supplemented with 10 g/l sucrose (MS10) and 160 ml vermiculite. The seeds were left to germinate for 8 days at 25°C and 0.5 W/M^2 light. Eight day old cotyledon explants were pre-cultured for 24 hours in Petri dishes containing a two week old feeder layer of tobacco suspension cells plated on co-cultivation medium (MS30 pH 5.8 supplemented with Nitsch vitamines (Duchefa Biochemie BV, Haarlem, The Netherlands), 0.5 g/l MES buffer and 8 g/l Daichin agar).

[0054] Overnight cultures of *A. tumefaciens* were centrifuged and the pellet was resuspended in cell suspension medium (MS30 pH 5.8 supplemented with Nitsch vitamines, 0.5 g/l MES buffer, pH 5.8) containing 200 µM acetosyringone to a final $O.D._{600}$ of 0.25. The explants were then infected with the diluted overnight culture of *A. tumefaciens* strain UIA143 (Farrand et al., 1989) containing the helper plasmid pCH32 (Hamilton et al., 1996) and pRGC2-blb for 25 minutes, blotted dry on sterile filter paper and co-cultured for 48 hours on the original feeder layer plates. Culture conditions were as described above.

[0055] Following the co-cultivation, the cotyledons explants were transferred to Petri dishes with selective shoot inducing medium (MS pH 5.8 supplemented with 10 g/l glucose, including Nitsch vitamines, 0.5 g/l MES buffer, 5 g/l agargel, 2 mg/l zeatine riboside, 400 mg/l carbenicilline, 100 mg/l kanamycine, 0.1 mg/l IAA) and cultured at 25°C with 3-5 W/m^2 light. The explants were sub-cultured every 3 weeks onto fresh medium. Emerging shoots were dissected from the underlying callus and transferred to containers with selective root inducing medium (MS10 pH 5.8 supplemented with Nitsch vitamines, 0.5 g/l MES buffer, 5 g/l agargel, 0.25 mg/l IBA, 200 mg/l carbenicillin and 100 mg/l kanamycine).

Complementation of the susceptible phenotype in tomato

[0056] Primary transformants were tested for *P. infestans* resistance essentially as described in Example 1 for potato leaves, except that a *P. infestans* isolate was used that is specific for tomato. The tomato isolate was obtained from Plant Research International BV (Wageningen, The Netherlands). In total 10 transformants containing an intact

RGC2-blb construct were tested for resistance to *P. infestans*. The disease resistance assay revealed that *Rpi-blb* is able to complement a susceptible tomato phenotype.

Molecular analysis of primary transformants

RT-PCR analysis

[0057] In order to produce cDNA, a mix of 19 µl containing 1 µg of total or polyA RNA, 0.25 mM of each dNTP, 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT and 530 ng oligo d(T) primer, GCTGTCAACGATACGCTACGTAACGGCATGACAGTG$(T)_{18}$ was denatured (1 min 83°C). Subsequently, the mix was placed at 42°C and 1 µl reverse transcriptase (M-MLV reverse transcriptase, Promega Benelux b.v., Leiden, The Netherlands) was added. After 60 min, the mix was heated for 1 min at 99°C and transferred to ice. 2 µl cDNA was used for standard PCR.

Rapid amplification of cDNA ends

[0058] The 5' and 3' ends of the *Rpi-blb* cDNA were determined by rapid amplification of cDNA ends (RACE) using the GeneRacer™ kit (Invitrogen™, The Netherlands). 3' RACE was carried out with the primers GSP1 (5'-GAGGAATCCATCTCCCAGAG) and GSP2 (5'-GTGCTTGAAGAGATGATAATTCACGAG) in combination with the GeneRacer™ 3' primer and GeneRacer™ 3' nested primer. 5' RACE was carried out on cDNA synthesised with the primer GSP3 (5'-GTCCATCTCACCAAGTAGTGG) using primers GSP4 (5'-GAAATGCTCAGTAACTCTCTGG) and GSP5 (5'-GGAGGACTGAAAGGTGTTGG) in combination with the GeneRacer™ 5' primer and GeneRacer™ 5' nested primer (Figure 7).

EXAMPLE 6: STRUCTURE OF THE *Rpi-blb* GENE AND THE CORRESPONDING PROTEIN.

[0059] The size and structure of the *Rpi-blb* gene was determined by comparing the genomic sequence derived from the insert of pRGC2-blb with cDNA fragments generated by 5' and 3' rapid amplification of cDNA ends. RACE identified 5' and 3' *Rpi-blb* specific cDNA fragments of a single species, respectively, suggesting that the genomic clone encodes a single *Rpi-blb* specific transcript. The coding sequence of the *Rpi-blb* transcript is estimated to be 2910 nucleotides (nt). The *Rpi-blb* gene contains a single intron of 678 nt starting 428 nt after the translational ATG start codon of the gene (Figure 3C).

[0060] The deduced open reading frame of the *Rpi-blb* gene encodes a predicted polypeptide of 970 amino acids with an estimated molecular weight of 110.3 kD (Figure 8). Several functional motifs present in *R* genes of the NBS-LRR class of plant R genes are apparent in the encoded protein which can be subdivided into 3 domains (A, B and C; Figure 8). The N-terminal part of the protein contains potential coiled-coil domains, heptad repeats in which the first and fourth residues are generally hydrophobic (domain A). Domain B harbours the NBS and other motifs that constitute the NB-ARC domain (ARC for Apaf-1, R protein, and CED-4) of R proteins and cell death regulators in animals (van der Biezen and Jones, 1998). This domain includes the Ap-ATPase motifs present in proteins of eukaryotic and prokaryotic origin (Aravind et *al.*, 1999). The C-terminal half of Rpi-blb comprises a series of 20 irregular LRRs (domain C). The LRRs can be aligned according to the consensus sequence LxxLxxLxLxxC/N/SxxLxxLPxxa, where x designates any residue and "a" designates the positions of aliphatic amino acids, followed by a region of varying length. This repeat format approximates the consensus for cytoplasmic LRRs (Jones and Jones, 1997).

EXAMPLE 7: NATURAL HOMOLOGUES AND ARTIFICIAL VARIANTS OF THE *Rpi-blb* GENE

Natural homologues

[0061] BLASTX homology searches with the coding sequence of the *Rpi-blb* gene revealed that amino acid sequence homology with various state of the art genes does not exceed 36% sequence identity (Table 4). The best BLASTX score was obtained with an NBS-LRR gene derived from *Oryza saliva* (36% amino acid sequence identity). NBS-LRR genes sharing an overall sequence homology of 27-36% amino-acid sequence identity with *Rpi-blb* can be found among others in *Arabidopsis thaliana, Phaseolus vulgaris, Lycopersicon esculentum* (*Fusarium I2* gene cluster; Ori et al., 1997; Simons et al, 1998), *Zea mays, Hordeum vulgare* and *Lactuca sativa*. Phylogenetic studies of the deduced amino acid sequences of *Rpi-blb, RGC1-blb, RGC3-blb, RGC4-blb* and those of the most homologous state of the art genes (as defined by BLASTX) derived from diverse species, using the Neighbour-Joining method of Saitou and Nei (1987), shows that members of the *Rpi-blb* gene cluster can be placed in a separate branch (Figure 9).

[0062] Sequence comparisons of the four R gene candidates identified on 8005-8 BAC clone SPB4 show that sequence homology within the *Rpi-blb* gene cluster varies between 70% and 81% at the amino acid sequence level. The

deduced amino acid sequence of *Rpiblb* shares the highest overall homology with *RGC3-blb* (81% amino acid sequence identity; Table 4). When the different domains are compared it is clear that the putative effector domains present in the N-terminal halves of the proteins (coiled-coil and NB-ARC domains) share a higher degree of homology (91% amino acid sequence identity) than the C-terminal halves of these proteins which are thought to contain the recognition domains (LRRs; 71% amino acid sequence identity). Comparison of all four amino acid sequences revealed a total of 104 Rpi-blb specific amino acid residues (Figure 10). The majority of these are located in the LRR region (80/104). Within the latter region, these specific residues are concentrated in the LRR subdomain xxLxLxxxx. The relative frequency of these specific amino-acid residues within this LRR subdomain is more than two times higher (28.3%) than that observed in the rest of the LRR domain (12.3%). The residues positioned around the two conserved leucine residues in the consensus xxLxxLxxxx are thought to be solvent exposed and are therefore likely to be involved in creating/maintaining recognition specificity of the resistance protein.

[0063] Sequences of additional homologues of the *Rpi-blb* gene can be obtained by screening genomic DNA or insert libraries, e.g. BAC libraries with primers based on signature sequences of the *Rpi-blb* gene. Screening of various *Solanum* BAC libraries with primer sets A and/or B (Table 2 and Figure 7) identified numerous *Rpi-blb* homologues derived from different *Solanum* species. Alignment of these additional sequences with those presented in Figure 10 will help identify *Rpi-blb* homologues and specific amino-acid residues therein responsible for *P. infestans* resistance specificity. Furthermore, testing additional sequences in the above described phylogenetic tree analyses, e.g. using the Neighbour-Joining method of Saitou and Nei (1987), provides additional identification of genes belonging to the *Rpi-blb* gene cluster.

Artificial variants

[0064] Domain swaps between the different homologues can be made to ascertain the role of the different sequences in *P. infestans* resistance. The restriction enzyme *NsiI* for example, which recognises the DNA sequence ATGCAT present in the conserved MHD motif can be used to swap the complete LRR domain of *Rpi-blb* with that of *RGC1-blb* or *RGC3-blb* using techniques known to those skilled in the art. Chimeric variants of the *Rpi-blb* gene were made which encode the N-terminal half of Rpi-blb and the C-terminal half of RGC1-blb or RGC3-blb and visa versa, i.e., the N-terminal half of RGC1-blb or RGC3-blb and the C-terminal half of Rpi-blb (Figure 11). These variants were transformed to the susceptible potato genotype Impala and tested for *P. infestans* resistance. Chimeric *RGC3-blb* genes containing the LRR domain of *Rpi-blb* were resistant to *P. infestans* indicating that the specificity of the *Rpi-blb* gene is encoded by this part of the gene.

Table 1.

Overview of *P. infestans* susceptibility in different *S. bulbocastanum* accessions							
S. bulbocastanum accession			#	#	#	%	
CGN	BGRC	PI	plants	R	V	susceptibi lity	cluster[a]
17692	8005	275193	11	10	1	9	a
	8006	275194	16	15	1	6	a
17693	8008	275198	19	18		0	b
17687	7997	243505	35	25	4	14	b
17688	7999	255518	19	19	0	0	c

[a] The letters a, b and c represent relative geographical origins depicted in Figure 1

Table 2. Overview of markers used for mapping *Rpi-blb*

Marker	Ori[a]	Sequence[b]	Annealing temp (°C)	Restriction enzyme[c]
TG513	F	CGTAAACGCACCAAAAGCAG	58	a.s.
	R	GATTCAAGCCAGGAACCGAG		
TG330	F	CAGCTGCCACAGCTCAAGC	56	TaqI
	R	TACCTACATGTACAGTACTGC		
CT88	F	GGCAGAAGAGCTAGGAAGAG	57	MboI
	R	ATGGCGTGATACAATCCGAG		
	F	TTCAAGAGCTTGAAGACATAACA	60	a.s.
	R	ATGGCGTGATACAATCCGAG		
CT64	F	ACTAGAGGATAGATTCTTGG	56	CfoI
	R	CTGGATGCCTTTCTCTATGT		
B139R	F	GATCAGAAGTGCCTTGAACC	56	TaqI
	R	CAAGGAGCTTGGTCAGCAG		
SPB33L	F	ATTGCACAGGAGCAGATCTG	59	HinfI
	R	TGTAAGAGAGCAAGAGGCAC		
SPB42L	F	AGAGCAGTCTTGAAGGTTGG	58	CfoI
	R	GATGGTAACTAAGCCTCAGG		
B149R	F	GACAGATTTCTCATAAACCTGC	58	MseI / XbaI
	R	AATCGTGCATCACTAGAGCG		
RGC1-4	F	TGTGGAGTAAGAGAGGAAGG	62	SspI / MseI
	R	TCAGCTGAGCAGTGTGTGG		
A	F	ATGGCTGAAGCTTTCATTCAAGTTCTG	60	
	R	TCACACCGCTTGATCAGTTGTGGAC		
B	F	TRCATGAYCTMATCCATGATTTGC	60	
	R	GMAATTTTGTGCCAGTCTTCTCC		

[a] Orientation of the primer, F: forward, R: reverse

[b] primer sequences according to IUB codes

[c] a.s.: allele specific.

Table 3.

Phytophthora infestans resistance assays		
Genotype[a]	RGC-containing plants/ transformants	R plants / RGC-containing plants
R_0(RGC1-blb)	15/20[b]	0/15
R_0(RGC2-blb)	7/31[c]	6/7
Ro(RGC3-blb)	0/6[c]	-
	14/21[d]	0/14
R_0(RGC4-blb)	1/7[c]	0/1
	18/19[b]	0/18

[a] R_0 genotypes are primary transformants obtained from transformation of the susceptible potato cultivar Impala with a T-DNA constructs containing the *Rpi-blb* gene candidates *RGC1-blb, RGC2-blb, RGC3-blb* or *RGC4-blb. Agrobacteriumtumefaciens* strains AGLO[b], LBA4404[c], or UIA143[d] were used for transformation of the *P. infestans* susceptible potato cultivar Impala. Kan^R: Kanamycin resistant.

Table 4

Comparison of nucleotide and amino acid sequence homology									
		8005-8 BAC SPB4						Rice RGC	Arabidopsis RGC	Tomato I2C-1
		RGC3- blb		RGC1-*bld*		RGC4-*bld*				
Rpi-*bld*	nt [a]	88		84		81		-	-	-
	aa [a]	81		76		70		37	32	32
		N[b]	Cb	N	C	N	C			
		91	71	79	72	75	66			

[a] Percentage nucleotide (nt) and amino acid (aa) sequence identity.

[b] Separate comparisons were made for the N-terminal (N) and C-terminal (C) halves of the protein sequences. The border between the two halves is the conserved *NsiI* restriction site in the DNA sequence (position 1417 of the *Rpi-blb* coding sequence).

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Rpi-blb protein sequence domain A, B and C

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Arg Leu Ser Ser Met Phe Ser Thr Ile Gln Ala Val Leu Glu Asp Ala
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Gln Glu Lys Gln Leu Asn Asn Lys Pro Leu Glu Asn Trp Leu Gln Lys
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595 600 605

Leu Thr Cys Met Pro Pro Arg Ile Gly Ser Leu Thr Cys Leu Lys Thr
610 615 620

Leu Gly Gln Phe Val Val Gly Arg Lys Lys Gly Tyr Gln Leu Gly Glu
625 630 635 640

Leu Gly Asn Leu Asn Leu Tyr Gly Ser Ile Lys Ile Ser His Leu Glu
645 650 655

Arg Val Lys Asn Asp Lys Asp Ala Lys Glu Ala Asn Leu Ser Ala Lys
660 665 670

Gly Asn Leu His Ser Leu Ser Met Ser Trp Asn Asn Phe Gly Pro His
675 680 685

Ile Tyr Glu Ser Glu Glu Val Lys Val Leu Glu Ala Leu Lys Pro His
690 695 700

Ser Asn Leu Thr Ser Leu Lys Ile Tyr Gly Phe Arg Gly Ile His Leu
705 710 715 720

Pro Glu Trp Met Asn His Ser Val Leu Lys Asn Ile Val Ser Ile Leu
725 730 735

Ile Ser Asn Phe Arg Asn Cys Ser Cys Leu Pro Pro Phe Gly Asp Leu

740 745 750

Pro Cys Leu Glu Ser Leu Glu Leu His Trp Gly Ser Ala Asp Val Glu
755 760 765

Tyr Val Glu Glu Val Asp Ile Asp Val His Ser Gly Phe Pro Thr Arg
770 775 780

Ile Arg Phe Pro Ser Leu Arg Lys Leu Asp Ile Trp Asp Phe Gly Ser
785 790 795 800

Leu Lys Gly Leu Leu Lys Lys Glu Gly Glu Glu Gln Phe Pro Val Leu
805 810 815

Glu Glu Met Ile Ile His Glu Cys Pro Phe Leu Thr Leu Ser Ser Asn
820 825 830

Leu Arg Ala Leu Thr Ser Leu Arg Ile Cys Tyr Asn Lys Val Ala Thr
835 840 845

Ser Phe Pro Glu Glu Met Phe Lys Asn Leu Ala Asn Leu Lys Tyr Leu
850 855 860

Thr Ile Ser Arg Cys Asn Asn Leu Lys Glu Leu Pro Thr Ser Leu Ala
865 870 875 880

Ser Leu Asn Ala Leu Lys Ser Leu Lys Ile Gln Leu Cys Cys Ala Leu
885 890 895

Glu Ser Leu Pro Glu Glu Gly Leu Glu Gly Leu Ser Ser Leu Thr Glu
900 905 910

Leu Phe Val Glu His Cys Asn Met Leu Lys Cys Leu Pro Glu Gly Leu
915 920 925

Gln His Leu Thr Thr Leu Thr Ser Leu Lys Ile Arg Gly Cys Pro Gln
930 935 940

Leu Ile Lys Arg Cys Glu Lys Gly Ile Gly Glu Asp Trp His Lys Ile
945 950 955 960

Ser His Ile Pro Asn Val Asn Ile Tyr Ile
965 970

<210> 42
<211> 979
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: alignment RGC3-blb

<220>
<221> SITE
<222> (1)..(979)

<400> 42
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20 25 30

Arg Leu Ser Ser Met Phe Ser Thr Ile Gln Ala Val Leu Glu Asp Ala
35 40 45

Gln Glu Lys Gln Leu Asn Asp Lys Pro Leu Glu Asn Trp Leu Gln Lys
50 55 60

Leu Asn Ala Ala Thr Tyr Glu Val Asp Asp Ile Leu Asp Glu Tyr Lys
65 70 75 80

Thr Lys Ala Thr Arg Phe Leu Gln Ser Glu Tyr Gly Arg Tyr His Pro
85 90 95

Lys Val Ile Pro Phe Arg His Lys Val Gly Lys Arg Met Asp Gln Val
100 105 110

Met Lys Lys Leu Asn Ala Ile Ala Glu Glu Arg Lys Asn Phe His Leu
115 120 125

Gln Glu Lys Ile Ile Glu Arg Gln Ala Ala Thr Arg Glu Thr Gly Ser
130 135 140

Val Leu Thr Glu Pro Gln Val Tyr Gly Arg Asp Lys Glu Lys Asp Glu
145 150 155 160

Ile Val Lys Ile Leu Ile Asn Asn Val Ser Asp Ala Gln Lys Leu Ser
165 170 175

Val Leu Pro Ile Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu Ser
180 185 190

Gln Met Val Phe Asn Asp Gln Arg Val Thr Glu Arg Phe Tyr Pro Lys
195 200 205

Ile Trp Ile Cys Val Ser Asp Asp Phe Asp Glu Lys Arg Leu Ile Lys
210 215 220

Ala Ile Val Glu Ser Ile Glu Gly Lys Ser Leu Ser Asp Met Asp Leu
225 230 235 240

Ala Pro Leu Gln Lys Lys Leu Gln Glu Leu Leu Asn Gly Lys Arg Tyr
245 250 255

Phe Leu Val Leu Asp Asp Val Trp Asn Glu Asp Gln His Lys Trp Ala
260 265 270

Asn Leu Arg Ala Val Leu Lys Val Gly Ala Ser Gly Ala Phe Val Leu
275 280 285

Thr Thr Thr Arg Leu Glu Lys Val Gly Ser Ile Met Gly Thr Leu Gln
290 295 300

Pro Tyr Glu Leu Ser Asn Leu Ser Pro Glu Asp Cys Trp Phe Leu Phe
305 310 315 320

Met Gln Arg Ala Phe Gly His Gln Glu Glu Ile Asn Pro Asn Leu Val
325 330 335

Ala Ile Gly Lys Glu Ile Val Lys Lys Cys Gly Gly Val Pro Leu Ala
340 345 350

Ala Lys Thr Leu Gly Gly Ile Leu Arg Phe Lys Arg Glu Glu Arg Glu
355 360 365

Trp Glu His Val Arg Asp Ser Pro Ile Trp Asn Leu Pro Gln Asp Glu
370 375 380

Ser Ser Ile Leu Pro Ala Leu Arg Leu Ser Tyr His His Leu Pro Leu
385 390 395 400

Asp Leu Asp Gln Cys Phe Val Tyr Cys Ala Val Phe Pro Lys Asp Thr
405 410 415

Lys Met Ala Lys Glu Asn Leu Ile Ala Phe Trp Met Ala His Gly Phe
420 425 430

Leu Leu Ser Lys Gly Asn Leu Glu Leu Glu Asp Val Gly Asn Glu Val
435 440 445

Trp Asn Glu Leu Tyr Leu Arg Ser Phe Phe Gln Glu Ile Glu Val Glu
450 455 460

Ser Gly Lys Thr Tyr Phe Lys Met His Asp Leu Ile His Asp Leu Ala
465 470 475 480

Thr Ser Leu Phe Ser Ala Asn Thr Ser Ser Ser Asn Ile Arg Glu Ile
485 490 495

Asn Ala Asn Tyr Asp Gly Tyr Met Met Ser Ile Gly Phe Ala Glu Val
500 505 510

Val Ser Ser Tyr Ser Pro Ser Leu Leu Gln Lys Phe Val Ser Leu Arg
515 520 525

Val Leu Asn Leu Arg Asn Ser Asn Leu Asn Gln Leu Pro Ser Ser Ile
530 535 540

Gly Asp Leu Val His Leu Arg Tyr Leu Asp Leu Ser Gly Asn Phe Arg
545 550 555 560

Ile Arg Asn Leu Pro Lys Arg Leu Cys Lys Leu Gln Asn Leu Gln Thr
565 570 575

Leu Asp Leu His Tyr Cys Asp Ser Leu Ser Cys Leu Pro Lys Gln Thr
580 585 590

Ser Lys Leu Gly Ser Leu Arg Asn Leu Leu Leu Asp Gly Cys Ser Leu
595 600 605

Thr Ser Thr Pro Pro Arg Ile Gly Leu Leu Thr Cys Leu Lys Ser Leu
610 615 620

Ser Cys Phe Val Ile Gly Lys Arg Lys Gly Tyr Gln Leu Gly Glu Leu
625 630 635 640

Lys Asn Leu Asn Leu Tyr Gly Ser Ile Ser Ile Thr Lys Leu Asp Arg
645 650 655

Val Lys Lys Asp Ser Asp Ala Lys Glu Ala Asn Leu Ser Ala Lys Ala
660 665 670

Asn Leu His Ser Leu Cys Leu Ser Trp Asp Leu Asp Gly Lys His Arg
675 680 685

Tyr Asp Ser Glu Val Leu Glu Ala Leu Lys Pro His Ser Asn Leu Lys
690 695 700

Tyr Leu Glu Ile Asn Gly Phe Gly Gly Ile Arg Leu Pro Asp Trp Met
705 710 715 720

Asn Gln Ser Val Leu Lys Asn Val Val Ser Ile Arg Ile Arg Gly Cys
725 730 735

Glu Asn Cys Ser Cys Leu Pro Pro Phe Gly Glu Leu Pro Cys Leu Glu
740 745 750

Ser Leu Glu Leu His Thr Gly Ser Ala Asp Val Glu Tyr Val Glu Asp
755 760 765

Asn Val His Pro Gly Arg Phe Pro Ser Leu Arg Lys Leu Val Ile Trp

770 775 780

Asp Phe Ser Asn Leu Lys Gly Leu Leu Lys Lys Glu Gly Glu Glu Gln
785 790 795 800

Phe Pro Val Leu Glu Glu Met Thr Phe Tyr Trp Cys Pro Met Phe Val
805 810 815

Ile Pro Thr Leu Ser Ser Val Lys Thr Leu Lys Val Ile Ala Thr Asp
820 825 830

Ala Thr Val Leu Arg Ser Ile Ser Asn Leu Arg Ala Leu Thr Ser Leu
835 840 845

Asp Ile Ser Asn Asn Val Glu Ala Thr Ser Leu Pro Glu Glu Met Phe
850 855 860

Lys Ser Leu Ala Asn Leu Lys Tyr Leu Asn Ile Ser Phe Phe Arg Asn
865 870 875 880

Leu Lys Glu Leu Pro Thr Ser Leu Ala Ser Leu Asn Ala Leu Lys Ser
885 890 895

Leu Lys Phe Glu Phe Cys Asn Ala Leu Glu Ser Leu Pro Ala Glu Gly
900 905 910

Val Lys Gly Leu Thr Ser Leu Thr Glu Leu Ser Val Ser Asn Cys Met
915 920 925

Met Leu Lys Cys Leu Pro Glu Gly Leu Gln His Leu Thr Ala Leu Thr
930 935 940

Thr Leu Thr Ile Thr Gln Cys Pro Ile Val Phe Lys Arg Cys Glu Arg
945 950 955 960

Gly Ile Gly Glu Asp Trp His Lys Ile Ala His Ile Pro Tyr Leu Thr
965 970 975

Leu Tyr Glu

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<211> 992
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: alignment RGC1-blb

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<222> (1)..(992)

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20 25 30

Lys Leu Ser Ser Met Phe Ser Met Ile Gln Ala Val Leu Glu Asp Ala
35 40 45

Gln Glu Lys Gln Leu Lys Tyr Lys Ala Ile Lys Asn Trp Leu Gln Lys
50 55 60

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Leu Asn Val Ala Ala Tyr Glu Val Asp Asp Ile Leu Asp Asp Cys Lys
 65                  70                  75                  80

Thr Glu Ala Ala Arg Phe Lys Gln Ala Val Leu Gly Arg Tyr His Pro
            85                  90                  95

Arg Thr Ile Thr Phe Cys Tyr Lys Val Gly Lys Arg Met Lys Glu Met
        100                 105                 110

Met Glu Lys Leu Asp Ala Ile Ala Glu Glu Arg Arg Asn Phe His Leu
    115                 120                 125

Asp Glu Arg Ile Ile Glu Arg Gln Ala Ala Arg Arg Gln Thr Gly Phe
130                 135                 140

Val Leu Thr Glu Pro Lys Val Tyr Gly Arg Glu Lys Glu Glu Asp Glu
145                 150                 155                 160

Ile Val Lys Ile Leu Ile Asn Asn Val Ser Tyr Ser Glu Glu Val Pro
                165                 170                 175

Val Leu Pro Ile Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu Ala
            180                 185                 190

Gln Met Val Phe Asn Asp Gln Arg Ile Thr Glu His Phe Asn Leu Lys
        195                 200                 205

Ile Trp Val Cys Val Ser Asp Asp Phe Asp Glu Lys Arg Leu Ile Lys
    210                 215                 220

Ala Ile Val Glu Ser Ile Glu Gly Lys Ser Leu Gly Asp Met Asp Leu
225                 230                 235                 240

Ala Pro Leu Gln Lys Lys Leu Gln Glu Leu Leu Asn Gly Lys Arg Tyr
                245                 250                 255

Phe Leu Val Leu Asp Asp Val Trp Asn Glu Asp Gln Glu Lys Trp Asp
            260                 265                 270

Asn Leu Arg Ala Val Leu Lys Ile Gly Ala Ser Gly Ala Ser Ile Leu
        275                 280                 285

Ile Thr Thr Arg Leu Glu Lys Ile Gly Ser Ile Met Gly Thr Leu Gln
    290                 295                 300

Leu Tyr Gln Leu Ser Asn Leu Ser Gln Glu Asp Cys Trp Leu Leu Phe
305                 310                 315                 320

Lys Gln Arg Ala Phe Cys His Gln Thr Glu Thr Ser Pro Lys Leu Met
                325                 330                 335

Glu Ile Gly Lys Glu Ile Val Lys Lys Cys Gly Gly Val Pro Leu Ala
            340                 345                 350

Ala Lys Thr Leu Gly Gly Leu Leu Arg Phe Lys Arg Glu Glu Ser Glu
        355                 360                 365

Trp Glu His Val Arg Asp Ser Glu Ile Trp Asn Leu Pro Gln Asp Glu
    370                 375                 380

Asn Ser Val Leu Pro Ala Leu Arg Leu Ser Tyr His His Leu Pro Leu
385                 390                 395                 400

Asp Leu Arg Gln Cys Phe Ala Tyr Cys Ala Val Phe Pro Lys Asp Thr
                405                 410                 415

Lys Ile Glu Lys Glu Tyr Leu Ile Ala Leu Trp Met Ala His Ser Phe
            420                 425                 430
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Leu Leu Ser Lys Gly Asn Met Glu Leu Glu Asp Val Gly Asn Glu Val
435 440 445

Trp Asn Glu Leu Tyr Leu Arg Ser Phe Phe Gln Glu Ile Glu Val Lys
450 455 460

Ser Gly Lys Thr Tyr Phe Lys Met His Asp Leu Ile His Asp Leu Ala
465 470 475 480

Thr Ser Met Phe Ser Ala Ser Ala Ser Ser Arg Ser Ile Arg Gln Ile
485 490 495

Asn Val Lys Asp Asp Glu Asp Met Met Phe Ile Val Thr Asn Tyr Lys
500 505 510

Asp Met Met Ser Ile Gly Phe Ser Glu Val Val Ser Ser Tyr Ser Pro
515 520 525

Ser Leu Phe Lys Arg Phe Val Ser Leu Arg Val Leu Asn Leu Ser Asn
530 535 540

Ser Glu Phe Glu Gln Leu Pro Ser Ser Val Gly Asp Leu Val His Leu
545 550 555 560

Arg Tyr Leu Asp Leu Ser Gly Asn Lys Ile Cys Ser Leu Pro Lys Arg
565 570 575

Leu Cys Lys Leu Gln Asn Leu Gln Thr Leu Asp Leu Tyr Asn Cys Gln
580 585 590

Ser Leu Ser Cys Leu Pro Lys Gln Thr Ser Lys Leu Cys Ser Leu Arg
595 600 605

Asn Leu Val Leu Asp His Cys Pro Leu Thr Ser Met Pro Pro Arg Ile
610 615 620

Gly Leu Leu Thr Cys Leu Lys Thr Leu Gly Tyr Phe Val Val Gly Glu
625 630 635 640

Arg Lys Gly Tyr Gln Leu Gly Glu Leu Arg Asn Leu Asn Leu Arg Gly
645 650 655

Ala Ile Ser Ile Thr His Leu Glu Arg Val Lys Asn Asp Met Glu Ala
660 665 670

Lys Glu Ala Asn Leu Ser Ala Lys Ala Asn Leu His Ser Leu Ser Met
675 680 685

Ser Trp Asp Arg Pro Asn Arg Tyr Glu Ser Glu Glu Val Lys Val Leu
690 695 700

Glu Ala Leu Lys Pro His Pro Asn Leu Lys Tyr Leu Glu Ile Ile Asp
705 710 715 720

Phe Cys Gly Phe Cys Leu Pro Asp Trp Met Asn His Ser Val Leu Lys
725 730 735

Asn Val Val Ser Ile Leu Ile Ser Gly Cys Glu Asn Cys Ser Cys Leu
740 745 750

Pro Pro Phe Gly Glu Leu Pro Cys Leu Glu Ser Leu Glu Leu Gln Asp
755 760 765

Gly Ser Val Glu Val Glu Tyr Val Glu Asp Ser Gly Phe Leu Thr Arg
770 775 780

Arg Arg Phe Pro Ser Leu Arg Lys Leu His Ile Gly Gly Phe Cys Asn

785 790 795 800

Leu Lys Gly Leu Gln Arg Met Lys Gly Ala Glu Gln Phe Pro Val Leu
805 810 815

Glu Glu Met Lys Ile Ser Asp Cys Pro Met Phe Val Phe Pro Thr Leu
820 825 830

Ser Ser Val Lys Lys Leu Glu Ile Trp Gly Glu Ala Asp Ala Gly Gly
835 840 845

Leu Ser Ser Ile Ser Asn Leu Ser Thr Leu Thr Ser Leu Lys Ile Phe
850 855 860

Ser Asn His Thr Val Thr Ser Leu Leu Glu Glu Met Phe Lys Asn Leu
865 870 875 880

Glu Asn Leu Ile Tyr Leu Ser Val Ser Phe Leu Glu Asn Leu Lys Glu
885 890 895

Leu Pro Thr Ser Leu Ala Ser Leu Asn Asn Leu Lys Cys Leu Asp Ile
900 905 910

Arg Tyr Cys Tyr Ala Leu Glu Ser Leu Pro Glu Glu Gly Leu Glu Gly
915 920 925

Leu Ser Ser Leu Thr Glu Leu Phe Val Glu His Cys Asn Met Leu Lys
930 935 940

Cys Leu Pro Glu Gly Leu Gln His Leu Thr Thr Leu Thr Ser Leu Lys
945 950 955 960

Ile Arg Gly Cys Pro Gln Leu Ile Lys Arg Cys Glu Lys Gly Ile Gly
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980 985 990

<210> 44
<211> 1040
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: alignment RGC4-blb

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<221> SITE
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20 25 30

Lys Leu Ser Ser Val Phe Ser Thr Ile Gln Ala Val Leu Gln Asp Ala
35 40 45

Gln Glu Lys Gln Leu Lys Asp Lys Ala Ile Glu Asn Trp Leu Gln Lys
50 55 60

Leu Asn Ser Ala Ala Tyr Glu Val Asp Asp Ile Leu Gly Glu Cys Lys
65 70 75 80

Asn Glu Ala Ile Arg Phe Glu Gln Ser Arg Leu Gly Phe Tyr His Pro
85 90 95

Gly Ile Ile Asn Phe Arg His Lys Ile Gly Arg Arg Met Lys Glu Ile
100 105 110

Met Glu Lys Leu Asp Ala Ile Ser Glu Glu Arg Arg Lys Phe His Phe
115 120 125

Leu Glu Lys Ile Thr Glu Arg Gln Ala Ala Ala Ala Thr Arg Glu Thr
130 135 140

Val Gly Trp Gln Trp Gly Trp Ala Arg Leu Glu Tyr Lys Arg Leu Leu
145 150 155 160

Leu Gly Val Leu Met Arg Ile Met Ser Leu Arg Met His Val Ser Thr
165 170 175

Cys Ser Thr Leu Tyr Glu Phe Lys Phe Tyr Leu Cys Thr Pro Lys Val
180 185 190

Gly Ala Arg Arg Cys Phe Val Leu Thr Glu Pro Lys Val Tyr Gly Arg
195 200 205

Asp Lys Glu Glu Asp Glu Ile Val Lys Ile Leu Ile Asn Asn Val Asn
210 215 220

Val Ala Glu Glu Leu Pro Val Phe Pro Ile Ile Gly Met Gly Gly Leu
225 230 235 240

Gly Lys Thr Thr Leu Ala Gln Met Ile Phe Asn Asp Glu Arg Val Thr
245 250 255

Lys His Phe Asn Pro Lys Ile Trp Val Cys Val Ser Asp Asp Phe Asp
260 265 270

Glu Lys Arg Leu Ile Lys Thr Ile Ile Gly Asn Ile Glu Arg Ser Ser
275 280 285

Pro His Val Glu Asp Leu Ala Ser Phe Gln Lys Lys Leu Gln Glu Leu
290 295 300

Leu Asn Gly Lys Arg Tyr Leu Leu Val Leu Asp Asp Val Trp Asn Asp
305 310 315 320

Asp Leu Glu Lys Trp Ala Lys Leu Arg Ala Val Leu Thr Val Gly Ala
325 330 335

Arg Gly Ala Ser Ile Leu Ala Thr Thr Arg Leu Glu Lys Val Gly Ser
340 345 350

Ile Met Gly Thr Leu Gln Pro Tyr His Leu Ser Asn Leu Ser Pro His
355 360 365

Asp Ser Leu Leu Leu Phe Met Gln Arg Ala Phe Gly Gln Gln Lys Glu
370 375 380

Ala Asn Pro Asn Leu Val Ala Ile Gly Lys Glu Ile Val Lys Lys Cys
385 390 395 400

Gly Gly Val Pro Leu Ala Ala Lys Thr Leu Gly Gly Leu Leu Arg Phe
405 410 415

Lys Arg Glu Glu Ser Glu Trp Glu His Val Arg Asp Asn Glu Ile Trp
420 425 430

Ser Leu Pro Gln Asp Glu Ser Ser Ile Leu Pro Ala Leu Arg Leu Ser
435 440 445

Tyr His His Leu Pro Leu Asp Leu Arg Gln Cys Phe Ala Tyr Cys Ala
450 455 460

Val Phe Pro Lys Asp Thr Lys Met Ile Lys Glu Asn Leu Ile Thr Leu
465 470 475 480

Trp Met Ala His Gly Phe Leu Leu Ser Lys Gly Asn Leu Glu Leu Glu
485 490 495

Asp Val Gly Asn Glu Val Trp Asn Glu Leu Tyr Leu Arg Ser Phe Phe
500 505 510

Gln Glu Ile Glu Ala Lys Ser Gly Asn Thr Tyr Phe Lys Ile His Asp
515 520 525

Leu Ile His Asp Leu Ala Thr Ser Leu Phe Ser Ala Ser Ala Ser Cys
530 535 540

Gly Asn Ile Arg Glu Ile Asn Val Lys Asp Tyr Lys His Thr Val Ser
545 550 555 560

Ile Gly Phe Ala Ala Val Val Ser Ser Tyr Ser Pro Ser Leu Leu Lys
565 570 575

Lys Phe Val Ser Leu Arg Val Leu Asn Leu Ser Tyr Ser Lys Leu Glu
580 585 590

Gln Leu Pro Ser Ser Ile Gly Asp Leu Leu His Leu Arg Tyr Leu Asp
595 600 605

Leu Ser Cys Asn Asn Phe Arg Ser Leu Pro Glu Arg Leu Cys Lys Leu
610 615 620

Gln Asn Leu Gln Thr Leu Asp Val His Asn Cys Tyr Ser Leu Asn Cys
625 630 635 640

Leu Pro Lys Gln Thr Ser Lys Leu Ser Ser Leu Arg His Leu Val Val
645 650 655

Asp Gly Cys Pro Leu Thr Ser Thr Pro Pro Arg Ile Gly Leu Leu Thr
660 665 670

Cys Leu Lys Thr Leu Gly Phe Phe Ile Val Gly Ser Lys Lys Gly Tyr
675 680 685

Gln Leu Gly Glu Leu Lys Asn Leu Asn Leu Cys Gly Ser Ile Ser Ile
690 695 700

Thr His Leu Glu Arg Val Lys Asn Asp Thr Asp Ala Glu Ala Asn Leu
705 710 715 720

Ser Ala Lys Ala Asn Leu Gln Ser Leu Ser Met Ser Trp Asp Asn Asp
725 730 735

Gly Pro Asn Arg Tyr Glu Ser Lys Glu Val Lys Val Leu Glu Ala Leu
740 745 750

Lys Pro His Pro Asn Leu Lys Tyr Leu Glu Ile Ile Ala Phe Gly Gly
755 760 765

Phe Arg Phe Pro Ser Trp Ile Asn His Ser Val Leu Glu Lys Val Ile
770 775 780

Ser Val Arg Ile Lys Ser Cys Lys Asn Cys Leu Cys Leu Pro Pro Phe
785 790 795 800

Gly Glu Leu Pro Cys Leu Glu Asn Leu Glu Leu Gln Asn Gly Ser Ala

```
                    805                 810                 815
Glu Val Glu Tyr Val Glu Glu Asp Asp Val His Ser Arg Phe Ser Thr
            820                 825                 830

Arg Arg Ser Phe Pro Ser Leu Lys Lys Leu Arg Ile Trp Phe Phe Arg
        835                 840                 845

Ser Leu Lys Gly Leu Met Lys Glu Glu Gly Glu Glu Lys Phe Pro Met
    850                 855                 860

Leu Glu Glu Met Ala Ile Leu Tyr Cys Pro Leu Phe Val Phe Pro Thr
865                 870                 875                 880

Leu Ser Ser Val Lys Lys Leu Glu Val His Gly Asn Thr Asn Thr Arg
                885                 890                 895

Gly Leu Ser Ser Ile Ser Asn Leu Ser Thr Leu Thr Ser Leu Arg Ile
            900                 905                 910

Gly Ala Asn Tyr Arg Ala Thr Ser Leu Pro Glu Glu Met Phe Thr Ser
        915                 920                 925

Leu Thr Asn Leu Glu Phe Leu Ser Phe Phe Asp Phe Lys Asn Leu Lys
    930                 935                 940

Asp Leu Pro Thr Ser Leu Thr Ser Leu Asn Ala Leu Lys Arg Leu Gln
945                 950                 955                 960

Ile Glu Ser Cys Asp Ser Leu Glu Ser Phe Pro Glu Gln Gly Leu Glu
                965                 970                 975

Gly Leu Thr Ser Leu Thr Gln Leu Phe Val Lys Tyr Cys Lys Met Leu
            980                 985                 990

Lys Cys Leu Pro Glu Gly Leu Gln His Leu Thr Ala Leu Thr Asn Leu
        995                1000                1005

Gly Val Ser Gly Cys Pro Glu Val Glu Lys Arg Cys Asp Lys Glu Ile
   1010                1015                1020

Gly Glu Asp Trp His Lys Ile Ala His Ile Pro Asn Leu Asp Ile His
1025                1030                1035                1040
```

Claims

1. An isolated or recombinant nucleic acid or functional fragment thereof corresponding to one of a cluster of genes identifiable by phylogenetic tree analyses as corresponding to the *Rpi-blb, RGC1-blb, RGC3-blb and RGC4-blb* cluster of figure 9.

2. A nucleic acid according to claim 1 said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* family with resistance against an oomycete pathogen, or a functional equivalent thereof.

3. A nucleic acid according to claim 1 or 2 wherein said member of the *Solanacae* family comprises *S. tuberosum.*

4. A nucleic acid according to claim 1 to 3 where said resistance is race non-specific.

5. A nucleic acid according to claim 1 to 4 comprising a sequence as depicted in figure 6 for Rpi-blb or part thereof.

6. A nucleic acid according to claim 1 to 5 at least comprising a LRR domain.

7. A vector comprising a nucleic acid according to anyone of claims 1 to 6.

8. A host cell comprising a nucleic acid according to anyone of claims 1 to 6 or a vector according to claim 7.

9. A cell according to claim 8 comprising a plant cell.

10. A cell according to claim 9 wherein said plant comprises a member of the *Solanacae* family.

11. A plant comprising a cell according to anyone of claims 6 to 10.

12. A part derived from a plant according to claim 11.

13. A part according to claim 12 wherein said tuber comprises a potato or said fruit comprises a tomato.

14. Progeny of a plant according to claim 11.

15. A proteinaceous substance exhibiting the characteristic of providing at least partial resistance to an oomycete infection such as caused by a *Phytophthora* species when incorporated and expressed in a plant or plant cell.

16. A proteinaceous substance encoded by a nucleic acid according to anyone of claims 1 to 6.

17. A proteinaceous substance comprising an amino acid sequence as depicted in figure 8 or part thereof.

18. A binding molecule directed at a substance according to anyone of claims 15 to 17.

19. A binding molecule according to claim 18 comprising an antibody or fragment thereof.

20. A binding molecule directed at a nucleic acid according to anyone of claim 1 to 6.

21. A binding molecule according to claim 20 comprising a probe or primer.

22. A binding molecule according to anyone of claims 18 to 21 provided with a label.

23. A binding molecule according to claim 22 wherein said label comprises an excitable moiety.

24. Use of a nucleic acid according to anyone of claims 1 to 6 or a vector according to claim 7 or a cell according to anyone of claims 8 to 11 or a substance according to anyone of claims 15 to 17 or a binding molecule according to anyone of claims 18 to 23 in a method for providing a plant or its progeny with resistance against an oomycete infection.

25. Use according to claim 24 wherein said oomycete comprises *Phytophthora infestans*.

26. Use according to claim 24 or 25 wherein said plant comprises S. *tuberosum*.

27. A method for providing a plant or its progeny with at least partial resistance against an oomycete infection comprising providing said plant or part thereof with a gene or functional fragment thereof comprising a nucleic acid corresponding to one of a cluster of genes identifiable by phylogenetic tree analyses as corresponding to the *Rpi-blb, RCG1-blb, RCG3-blb* and *RCG4-blb*cluster of figure 9, said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* with resistance against an oomycete fungus, or providing said plant or part thereof with a nucleic acid according to anyone of claims 1 to 4 or a vector according to claim 5 or a cell according to claim 6 or a substance according to anyone of claims 15 to 18.

28. A method for selecting a plant or plant material or progeny thereof for its susceptibility or resistance to an oomycete infection comprising testing at least part of said plant or plant material or progeny thereof for the presence or absence of a nucleic acid corresponding to one of a cluster of genes identifiable by phylogenetic tree analyses as corresponding to the *Rpi-blb, RCG1-blb, RCG3-blb* and *RCG4-blb*cluster of figure 9, said nucleic acid encoding a gene product that is capable of providing a member of the *Solanacae* with resistance against an oomycete fungus.

29. A method according to claim 28 comprising contacting at least part of said plant or plant material or progeny thereof with a binding molecule according to anyone of claims 19 to 23 and determining the binding of said molecule to said part.

30. A method according to claim 29 wherein said oomycete comprises *Phytophthora infestans*.

31. A method according to claim 28 or 29 wherein said plant comprises *S. tuberosum*.

32. An isolated *S. bulbocastanum*, or part thereof, susceptible to an oomycete infection caused by *Phytophthora infestans*.

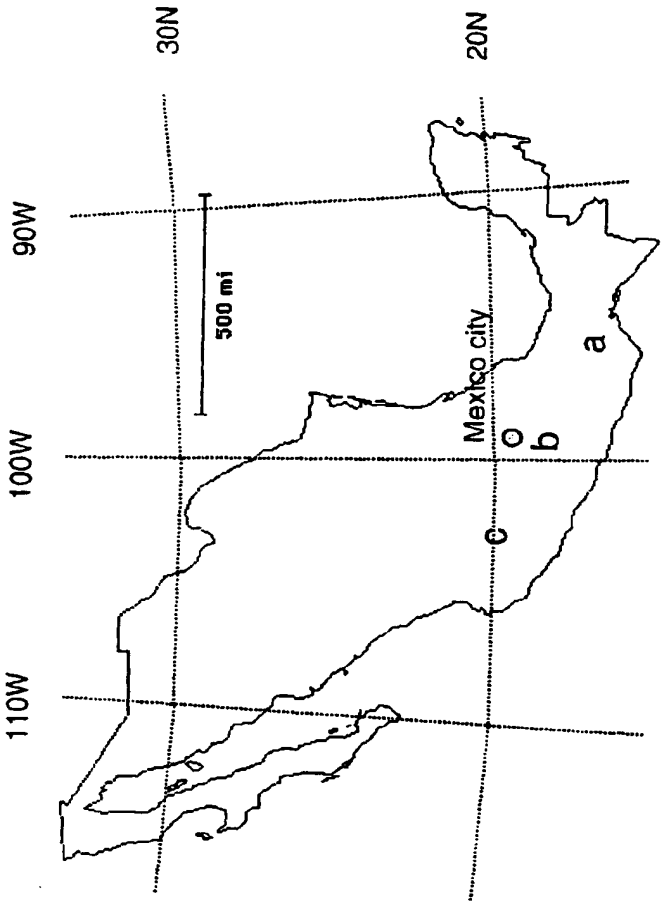

Figure 1

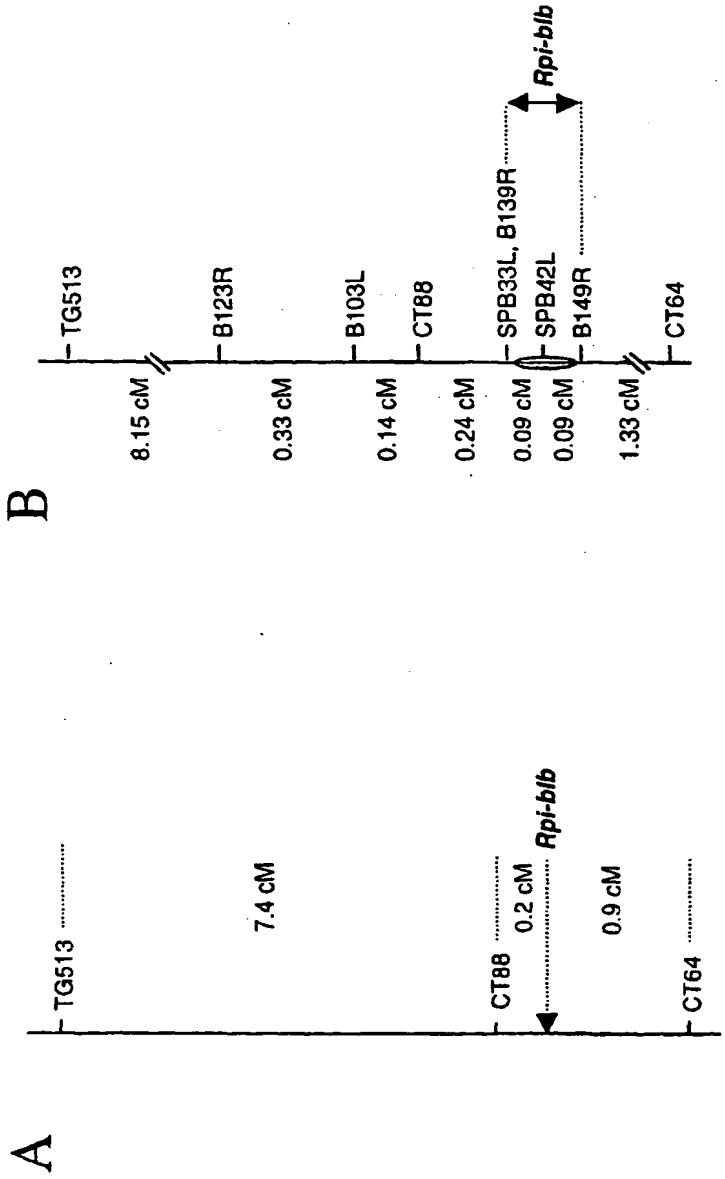

Figure 2

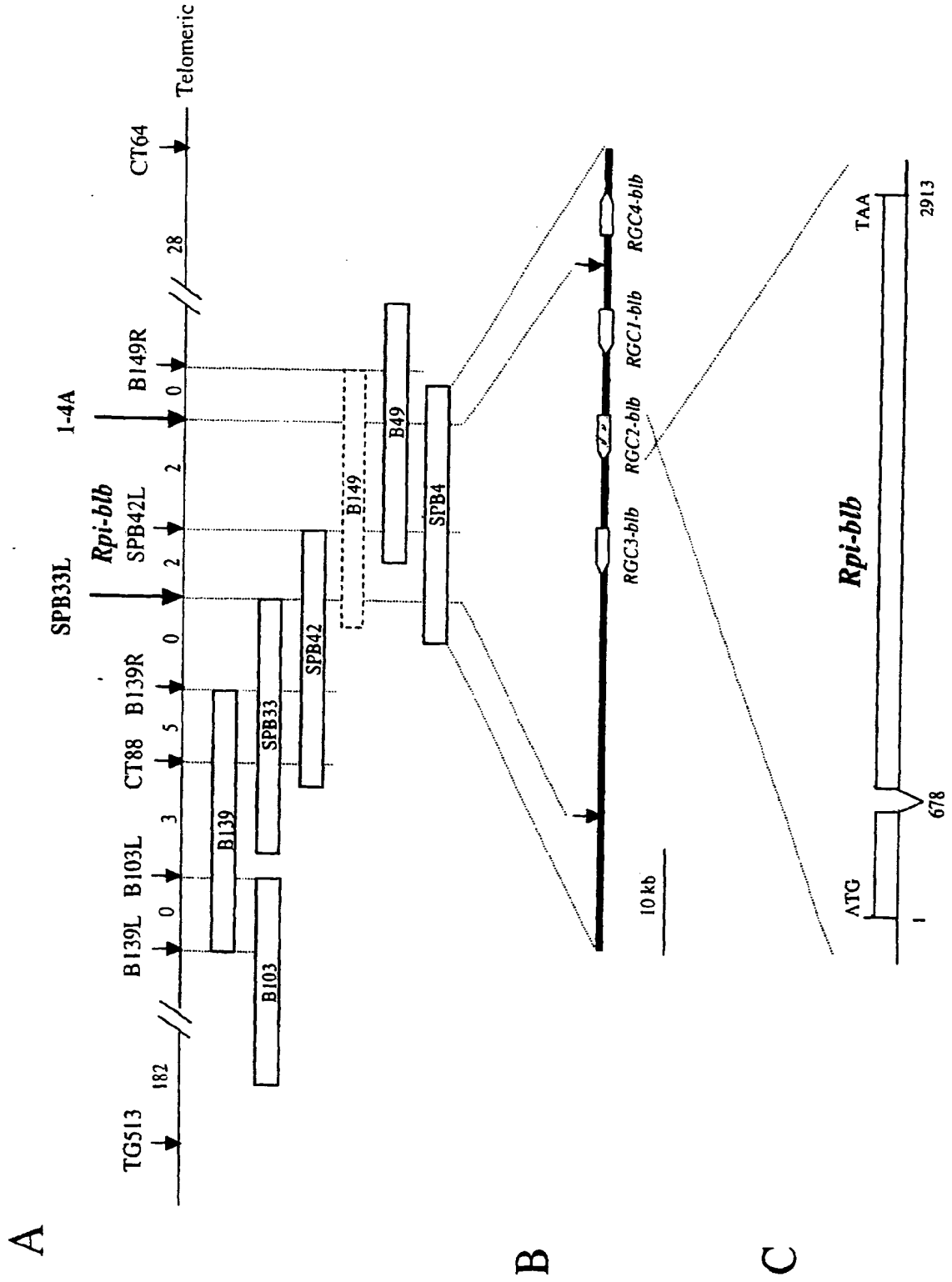

Figure 3

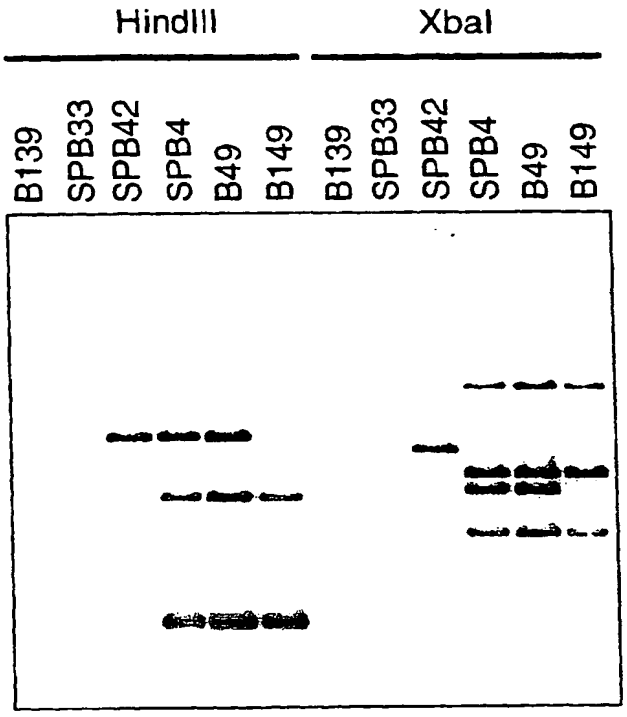

Figure 4

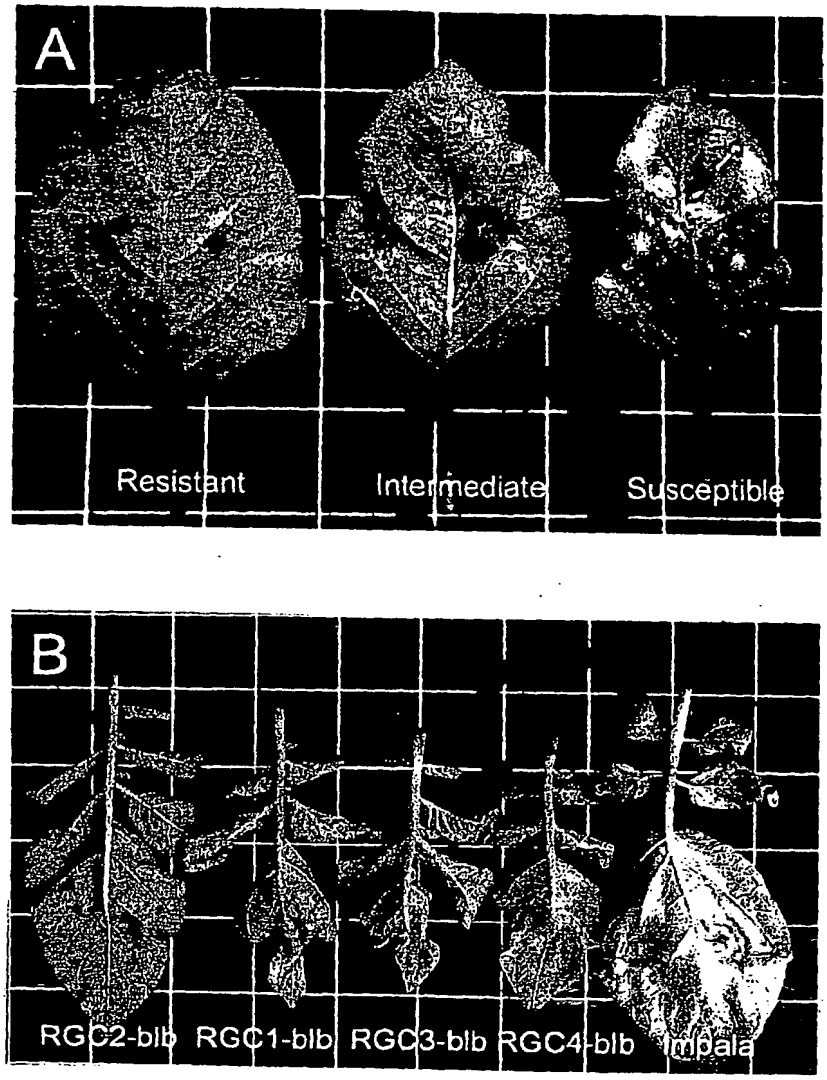

Figure 5

Figure 6A

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1       ATGGCTGAAGCTTTCATTCAAGTTCTGCTAGACAATCTCACTTCTTTCCT
51      CAAAGGGGAACTTGTATTGCTTTTCGGTTTTCAAGATGAGTTCCAAAGGC
101     TTTCAAGCATGTTTTCTACAATTCAAGCCGTCCTTGAAGATGCTCAGGAG
151     AAGCAACTCAACAACAAGCCTCTAGAAAATTGGTTGCAAAAACTCAATGC
201     TGCTACATATGAAGTCGATGACATCTTGGATGAATATAAAACCAAGGCCA
251     CAAGATTCTCCCAGTCTGAATATGGCCGTTATCATCCAAAGGTTATCCCT
301     TTCCGTCACAAGGTCGGGAAAAGGATGGACCAAGTGATGAAAAAACTAAA
351     GGCAATTGCTGAGGAAAGAAAGAATTTTCATTTGCACGAAAAAATTGTAG
401     AGAGACAAGCTGTTAGACGGGAAACAGGTTCTGTATTAACCGAACCGCAG
451     GTTTATGGAAGAGACAAAGAGAAAGATGAGATAGTGAAAATCCTAATAAA
501     CAATGTTAGTGATGCCCAACACCTTTCAGTCCTCCCAATACTTGGTATGG
551     GGGGATTAGGAAAAACGACTCTTGCCCAAATGGTCTTCAATGACCAGAGA
601     GTTACTGAGCATTTCCATTCCAAAATATGGATTTGTGTCTCGGAAGATTT
651     TGATGAGAAGAGGTTAATAAAGGCAATTGTAGAATCTATTGAAGGAAGGC
701     CACTACTTGGTGAGATGGACTTGGCTCCACTTCAAAAGAAGCTTCAGGAG
751     TTGCTGAATGGAAAAAGATACTTGCTTGTCTTAGATGATGTTTGGAATGA
801     AGATCAACAGAAGTGGGCTAATTTAAGAGCAGTCTTGAAGGTTGGAGCAA
851     GTGGTGCTTCTGTTCTAACCACTACTCGTCTTGAAAAGGTTGGATCAATT
901     ATGGGAACATTGCAACCATATGAACTGTCAAATCTGTCTCAAGAAGATTG
951     TTGGTTGTTGTTCATGCAACGTGCATTTGGACACCAAGAAGAAATAAATC
1001    CAAACCTTGTGGCAATCGGAAAGGAGATTGTGAAAAAAAGTGGTGGTGTG
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1101 AAGAGCATGGGAACATGTGAGAGACAGTCCGATTTGGAATTTGCCTCAAG

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1201 CTTGATTTGAAACAATGCTTTGCGTATTGTGCGGTGTTCCCAAAGGATGC

1251 CAAAATGGAAAAAGAAAAGCTAATCTCTCTCTGGATGGCGCATGGTTTTC

1301 TTTTATCAAAAGGAAACATGGAGCTAGAGGATGTGGGCGATGAAGTATGG

1351 AAAGAATTATACTTGAGGTCTTTTTTTCCAAGAGATTGAAGTTAAAGATGG

1401 TAAAACTTATTTCAAGATGCATGATCTCATCCATGATTTGGCAACATCTC

1451 TGTTTTCAGCAAACACATCAAGCAGCAATATCCGTGAAATAAATAAACAC

1501 AGTTACACACATATGATGTCCATTGGTTTCGCCGAAGTGGTGTTTTTTTA

1551 CACTCTTCCCCCCTTGGAAAAGTTTATCTCGTTAAGAGTGCTTAATCTAG

1601 GTGATTCGACATTTAATAAGTTACCATCTTCCATTGGAGATCTAGTACAT

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1701 GTTATGCAAGCTTCAAAATCTGCAAACTCTTGATCTACAATATTGCACCA

1751 AGCTTTGTTGTTTGCCAAAAGAAACAAGTAAACTTGGTAGTCTCCGAAAT

1801 CTTTTACTTGATGGTAGCCAGTCATTGACTTGTATGCCACCAAGGATAGG

1851 ATCATTGACATGCCTTAAGACTCTAGGTCAATTTGTTGTTGGAAGGAAGA

1901 AAGGTTATCAACTTGGTGAACTAGGAAACCTAAATCTCTATGGCTCAATT

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2001 CAATTTATCTGCAAAAGGGAATCTGCATTCTTTAAGCATGAGTTGGAATA

2051 ACTTTGGACCACATATATATGAATCAGAAGAAGTTAAAGTGCTTGAAGCC

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2151 AATCCATCTCCCAGAGTGGATGAATCACTCAGTATTGAAAAATATTGTCT

2201 CTATTCTAATTAGCAACTTCAGAAACTGCTCATGCTTACCACCCTTTGGT

2251 GATCTGCCTTGTCTAGAAAGTCTAGAGTTACACTGGGGGTCTGCGGATGT

2301 GGAGTATGTTGAAGAAGTGGATATTGATGTTCATTCTGGATTCCCCACAA

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2451 AGAGATGATAATTCACGAGTGCCCTTTTCTGACCCTTTCTTCTAATCTTA

2501 GGGCTCTTACTTCCCTCAGAATTTGCTATAATAAAGTAGCTACTTCATTC

2551 CCAGAAGAGATGTTCAAAAACCTTGCAAATCTCAAATACTTGACAATCTC

2601 TCGGTGCAATAATCTCAAAGAGCTGCCTACCAGCTTGGCTAGTCTGAATG

2651 CTTTGAAAAGTCTAAAAATTCAATTGTGTTGCGCACTAGAGAGTCTCCCT

2701 GAGGAAGGGCTGGAAGGTTTATCTTCACTCACAGAGTTATTTGTTGAACA

2751 CTGTAACATGCTAAAATGTTTACCAGAGGGATTGCAGCACCTAACAACCC

2801 TCACAAGTTTAAAAATTCGGGGATGTCCACAACTGATCAAGCGGTGTGAG

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2901 TATATATATTTAA

Figure 6B

```
1       ATGGCTGAAGCTTTCATTCAAGTTCTGCTAGACAATCTCACTTCTTTCCT
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101     TTTCAAGCATGTTTTCTACAATTCAAGCCGTCCTTGAAGATGCTCAGGAG
151     AAGCAACTCAACAACAAGCCTCTAGAAAATTGGTTGCAAAAACTCAATGC
201     TGCTACATATGAAGTCGATGACATCTTGGATGAATATAAAACCAAGGCCA
251     CAAGATTCTCCCAGTCTGAATATGGCCGTTATCATCCAAAGGTTATCCCT
301     TTCCGTCACAAGGTCGGGAAAAGGATGGACCAAGTGATGAAAAAACTAAA
351     GGCAATTGCTGAGGAAAGAAAGAATTTTCATTTGCACGAAAAAATTGTAG
401     AGAGACAAGCTGTTAGACGGGAAACAGGTACTCATCTTAAATTAGTATTA
451     CAACAACTAAGTTTATATTCATTTTTTTGGCAATTATCAAATTCAGAAAA
501     GGGTTAAATATACTCATGTCCTATCGTAAATAGTGTATATATACCTCTCG
551     TTGTACTTTCGATCTGAATATACTTGTCAAATCTGGCAAGCTCAGAATCA
601     AATTATCCACCCCAACTTTTAAATACTCGATATCTTTAGAAATCCACCTG
651     TCTAACTCATCCACTACCCATTCCCTTTGCTTTGAATTCTTTTCTTTACC
701     TATAAACTTGGAACACTCGATCCGTTTTGCTTTTCTTAACAAAGCAGCTC
751     AGAGAAAAGAGGTTTTCTTCTATTCTGTTTCTCTGTGTGCTGCACTTGGG
801     TCCTTAATCCCATTAAAAACAGGGCATGTTAATCCCAACGACGGTAGCCT
851     TTCCTGACAGCTGACTGTAAATTTTGTCTAACAAAGAAAAAAAAAGATTA
901     GACATGTTTTTCCTTGTCATTGATTAGGCTGGATTTCTTTCAGAGTGGAA
951     CATAGGGGATATATTGGACCAAAAGTAGAATGGGTATATATTTAAAGTAT
1001    TTCTGATAGAACAGGAGTATATTGTGCGAAAATATCCTCTATTTTCTGTT
1051    GTCTCCTAATGAGTTTGAATGTAATAATATTCTCATGTGGACATTGCTTG
1101    CACCAGGTTCTGTATTAACCGAACCGCAGGTTTATGGAAGAGACAAAGAG
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1201    CCTTTCAGTCCTCCCAATACTTGGTATGGGGGGATTAGGAAAAACGACTC
1251    TTGCCCAAATGGTCTTCAATGACCAGAGAGTTACTGAGCATTTCCATTCC
1301    AAAATATGGATTTGTGTCTCGGAAGATTTTGATGAGAAGAGGTTAATAAA
1351    GGCAATTGTAGAATCTATTGAAGGAAGGCCACTACTTGGTGAGATGGACT
1401    TGGCTCCACTTCAAAAGAAGCTTCAGGAGTTGCTGAATGGAAAAAGATAC
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2001 AGCTAGAGGATGTGGGCGATGAAGTATGGAAAGAATTATACTTGAGGTCT
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3351 AATTGTGTTGCGCACTAGAGAGTCTCCCTGAGGAAGGGCTGGAAGGTTTA
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3501 GATGTCCACAACTGATCAAGCGGTGTGAGAAGGGAATAGGAGAAGACTGG
3551 CACAAAATTTCTCACATTCCTAATGTGAATATATATATTTAA

Figure 6C

```
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6101 AGAGGGATTGCAGCACCTAACAACCCTCACAAGTTTAAAAATTCGGGGAT
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6201 AAAATTTCTCACATTCCTAATGTGAATATATATATTTAAGTTATTTGCTA

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7051 TCTCTAGGTAATTGATTTTACAGAAAAGGGCCAAAAATATCCCTGAAGTA
7101 CCAGAAAAGGTCTCAAAATACCAACCATCCACATTTTGGTCTAAAAATAT
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7251 TATTATGTGTCATTTTCTTATTGAATGAAATAAAAATCCACCTCTATTAA
7301 TTTTTTCCCATAATTTATCCAAATCAAAACAATATATTTTTTCAAGATC

Figure 6D

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151 AAGCAACTGAAGTACAAGGCAATAAAGAACTGGTTACAGAAACTCAATGT
201 TGCTGCATATGAAGTTGATGACATCTTGGATGACTGTAAAACTGAGGCAG
251 CAAGATTCAAGCAGGCTGTATTGGGGCGTTATCATCCACGGACCATCACT
301 TTCTGTTACAAGGTGGGAAAAAGAATGAAAGAAATGATGGAAAAACTAGA
351 TGCAATTGCAGAGGAACGGAGGAATTTTCATTTAGATGAAAGGATTATAG
401 AGAGACAAGCTGCTAGACGGCAAACAG**GTGCTCATCTTAATTTTATTTTA**
451 **AAACAAATAAGTATTACAAATTGCAGAGAAACGAAGGAATTTATATTCAT**
501 **TTTTATTTTTGGCAATTATCAAAGTCATTTGTGTTTTTAAGCTGGGGGGA**
551 **AGTTTCAAATATTTTCTCTAGTCTTAATGTTTGTCTCACTCACTCAGCAT**
601 **GATTTTCTCAATCCTTCACTTCAACTCCCCCCTACTGTGCAAATATCTTC**
651 **TCTATTTTCTGTTGACTCCTAATGAGCTTGAATGTAACAACATTCTTGTT**
701 **TGGAGCAG**GTTTTGTTTTAACTGAGCCAAAAGTTTATGGAAGGGAAAAAG
751 AGGAGGATGAGATAGTGAAAATCTTGATAAACAATGTTAGTTATTCCGAA
801 GAAGTTCCAGTACTCCCAATACTTGGTATGGGGGGACTAGGAAAGACGAC
851 TCTAGCCCAAATGGTCTTCAATGATCAAAGAATTACTGAGCATTTCAATC
901 TAAAGATATGGGTTTGTGTCTCAGATGATTTTGATGAGAAGAGGTTGATT
951 AAGGCAATTGTAGAATCTATTGAAGGAAAGTCACTGGGTGACATGGACTT
1001 GGCTCCCCTCCAGAAAAAGCTTCAGGAGTTGTTGAATGGAAAAAGATACT
1051 TTCTTGTTTTGGATGATGTTTGGAATGAAGATCAAGAAAAGTGGGATAAT
1101 CTTAGAGCAGTATTGAAGATTGGAGCTAGTGGTGCTTCAATTCTAATTAC
1151 TACTCGTCTTGAAAAAATTGGATCAATTATGGGAACTTTGCAACTATATC
1201 AGTTATCAAATTTGTCTCAAGAAGATTGTTGGTTGTTGTTCAAGCAACGT
1251 GCATTTTGCCACCAAACCGAAACAAGTCCTAAACTTATGGAAATCGGAAA
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1801 TTGTAACAAATTATAAAGATATGATGTCCATTGGTTTCTCCGAAGTGGTG
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1951 TAGTACATTTAAGATACCTTGACCTGTCTGGTAATAAAATTTGTAGTCTT
2001 CCAAAGAGGTTGTGCAAGCTTCAAAATCTGCAGACTCTTGATCTATATAA
2051 TTGCCAGTCACTTTCTTGTTTGCCGAAACAAACAAGTAAGCTTTGTAGTC
2101 TCCGGAATCTTGTACTTGATCACTGTCCATTGACTTCTATGCCACCAAGA
2151 ATAGGATTGTTGACATGCCTTAAGACACTAGGTTACTTTGTTGTAGGCGA
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2401 TCAAACCACATCCCAATCTGAAATATTTAGAAATCATTGACTTCTGTGGA
2451 TTCTGTCTCCCTGACTGGATGAATCACTCAGTTTTGAAAAATGTTGTCTC
2501 TATTCTAATTAGCGGTTGTGAAAACTGCTCGTGCTTACCACCCTTTGGTG
2551 AGCTGCCTTGTCTAGAAAGTCTGGAGTTACAAGACGGGTCTGTGGAGGTG
2601 GAGTATGTTGAAGATTCTGGATTCCTGACAAGAAGAAGATTTCCATCCCT
2651 GAGAAAACTTCATATAGGTGGCTTTTGTAATCTGAAAGGATTGCAGAGAA
2701 TGAAAGGAGCAGAGCAATTCCCCGTGCTTGAAGAGATGAAGATTTCGGAT
2751 TGCCCTATGTTTGTTTTTCCGACCCTTTCTTCTGTCAAGAAATTAGAAAT
2801 TTGGGGGGAGGCAGATGCAGGAGGTTTGAGCTCCATATCTAATCTCAGCA
2851 CTCTTACATCCCTCAAGATTTTCAGTAACCACACAGTGACTTCACTACTG
2901 GAAGAGATGTTCAAAAACCTTGAAAATCTCATATACTTGAGTGTCTCTTT
2951 CTTGGAGAATCTCAAAGAGCTGCCTACCAGCCTGGCTAGTCTCAACAATT
3001 TGAAGTGTCTGGATATTCGTTATTGTTACGCACTAGAGAGTCTCCCCGAG

3051 GAAGGGCTGGAAGGTTTATCTTCACTCACAGAGTTATTTGTTGAACACTG

3101 TAACATGCTAAAATGTTTACCAGAGGGATTGCAGCACCTAACAACCCTCA

3151 CAAGTTTAAAAATTCGGGGATGTCCACAACTGATCAAGCGGTGTGAGAAG

3201 GGAATAGGAGAAGACTGGCACAAAATTTCTCACATTCCTAATGTGAATAT

3251 ATATATTTAA

Figure 6E

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1     ATGGCTGAAGCTTTCATTCAAGTTGTGCTAGACAATCTCACTTCTTTCCT
51    CAAAGGGGAACTTGTATTGCTTTTCGGTTTTCAAGATGAGTTCCAAAGGC
101   TTTCAAGCATGTTTTCTACAATCCAAGCCGTCCTTGAAGATGCTCAAGAG
151   AAGCAACTCAACGACAAGCCTCTAGAAAATTGGTTGCAAAAACTCAATGC
201   TGCTACATATGAAGTCGATGACATCTTGGATGAATATAAAACTAAGGCCA
251   CAAGATTCTTGCAGTCTGAATATGGCCGTTATCATCCAAAGGTTATCCCT
301   TTCCGTCACAAGGTTGGGAAAAGGATGGACCAAGTGATGAAAAAACTGAA
351   TGCAATTGCTGAGGAACGAAAGAATTTTCATTTGCAAGAAAAGATTATAG
401   AGAGACAAGCTGCTACACGGGAAACAGGTACTCATCTTAAATTAGTATTA
451   CAACTTAGTTTATATTCATTTGTTTTGGGCAATGATCAAATTATGTAAAG
501   GTCAAATATACTCATGTACTACTGAAAATAGTTTAAATATACCTCTAGTT
551   ATACTATTAGTACGAACATACTCCTCCCATATACTTTGGAACAAATATTC
601   CCTTAACGAAATAAGACACGTGAAAAGTTCAGATTCAAATTATCCACCCT
651   CAATTTTAAGATCTGATTTCTTTAGGAAACCACTCATCTCCTCCGTTTTG
701   AGTTCTTAACGAAGCAGCTCAGAGAAAAGAGGTTTTCTTCTGTTCTGTTT
751   CTGCTGCATTTGTGTCTTAATCCAATAACAAACAATACAAATTAATATTA
801   TGTTCACGATGAGGGTAGTCTTTCTAGCTAGACATGAACTGAGTGTAAAT
851   TTTGTTTTAAGGAAGAAAAAGAAATGATTAGGCTGGATTTCTTTCAGAGT
901   GGAATATAGGGGGATAAAGTTGGAGCATAGAGTTCCATCGTTTATTTCTT
951   TCCTTAAAGTAACAAGTTCAACAAAATGATATCAAGGTACGGTAATGGAA
1001  AATTATTAGACACGTCTAAACTACAAAAATGGAATAGAAACTTAAATTAT
1051  CAGTGACAATATCATCCTTTAATAAAGCTACCAAATTTAAATCATGATAC
1101  AGAGAAGAAACCAAAAAAATTAGGGGTGAATTATTTGATTCTATGCTTAT
1151  CACATGTCTTCCCATCAACATCAAAGGAAAAATTGTGCCAAAGTATAAAC
1201  GGTGCGGTATATTTGGATTGAAAGTAAAACAGGAGGATACATTTGGACTA
1251  AAAGTATAACAATAAGTATATTTGATCATTTTATGTATCAAATTCATGTG
1301  GTTTTTGGGGAGAAGGGAAGTTTCAATGTTTTCAATCTGCTCCTCATCTC
1351  ATCCATATCTCTTTATTGTGCAAAACCCTTCTCTATTTAACTATTTTCTG
1401  CCGACTCCTAATGAGCTTGAATGTAACAATATTCTCATCTGGACATTGCT
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1551 AAACTCTCAGTCCTCCCAATACTTGGTATGGGGGGACTAGGAAAGACAAC
1601 TCTTTCCCAAATGGTCTTCAATGATCAGAGAGTAACTGAGCGTTTCTATC
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1701 AAGGCAATAGTAGAATCTATTGAAGGGAAGTCCCTCAGTGACATGGACTT
1751 GGCTCCACTTCAAAAGAAGCTTCAAGAGTTGCTGAATGGAAAAAGATACT
1801 TCCTTGTCTTAGATGATGTTTGGAATGAAGATCAACATAAGTGGGCTAAT
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2001 GCATTTGGACACCAAGAAGAAATAAATCCAAACCTTGTGGCAATCGGAAA
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2601 TTTGTCTCATTAAGGGTGCTTAATCTAAGAAACTCGAACCTAAATCAATT
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3801 GTCAGTAACTGTATGATGCTAAAATGTTTACCGGAGGGATTGCAGCACCT
3851 AACAGCCCTCACAACTTTAACAATTACTCAATGTCCAATAGTATTCAAGC
3901 GGTGTGAGAGAGGAATAGGAGAAGACTGGCACAAAATTGCTCACATTCCA
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Figure 6F

1 ATGGCGGAAGCTTTTCTTCAAGTTCTGCTAGAAAATCTCACTTCTTTCAT
51 CGGAGATAAACTTGTATTGATTTTCGGTTTCGAAAAGGAATGTGAAAAGC
101 TGTCGAGTGTGTTTTCCACAATTCAAGCTGTGCTTCAAGATGCTCAGGAG
151 AAGCAATTGAAGGACAAGGCAATTGAGAATTGGTTGCAGAAACTCAATTC
201 TGCTGCCTATGAAGTTGATGATATATTGGGCGAATGTAAAAATGAGGCAA
251 TAAGATTTGAGCAGTCTCGATTAGGGTTTTATCACCCAGGGATTATCAAT
301 TTCCGTCACAAAATTGGGAGAAGGATGAAAGAGATAATGGAGAAACTAGA
351 TGCAATATCTGAGGAAAGAAGGAAGTTTCATTTCCTTGAAAAAATTACAG
401 AGAGACAAGCTGCCGCTGCTACGCGTGAAACAG**GTGTGAGTACTGAGTAA**
451 **TTGTAGCTTAGTTAATATTCAATTTGTTACCACATCATGTGTTCACCGTG**
501 **ATCTCTACAG**TAGGATGGCAATGGGGCTGGGCGAGGTTGGAG**GTGTGCAG**
551 **GTGTGTGGCGCAACCCCAACTTTGAGTCTACATAAGTAGGTACTTAAATT**
601 **TGTATAGAGTTGAACAAG**TACAAACGCCTCCTACTTGGTGTCCTTATGCG
651 TATTATGTCACTTAGGATGCATGTGTCTACTTGTTCAACTTTATATGAGT
701 TTAAGTTCTACTTGTGCACACCCAAAGTTGGAGCGCGTAGAT**GTCAGTTG**
751 **ATACCAAGTTAAAAAGGCATATTTATGAATTATGCCTTTAAATTATGATT**
801 **CAATTTTGTATCAGTCTGTCCAAAATATGTTCTAGTGAAAGTGTTAAACT**
851 **TAGTCTGGATCTGCTATTGAAAGTGAATTTTTGTGGCACTAAACAATGCA**
901 **ATGGGTCTGGATTCATTTTTGCATTAACTTTTGTTTAGACGATTTTCTTT**
951 **ATCGAATTTTACTGTCTAAAATGGAAAAAGCAAAGAAATAAGAAGTATAC**
1001 **AGAGGCTGACTTCTTCATAGTATCTATCATATAAAAAAAAGCATTGATTA**
1051 **CTAGGATATGGGTTCTTTTAAATTACAAATTTGTGAGTTAAAACAGTTCT**
1101 **GTTGGGAAGGATTTAGATACACGTGGATAGTATCTAGAAGTTTTTTAAAT**
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1201 **TGCAAGGCTTGGCGGGTCGAAATCTTTGCAAGTTTGTGTGGGTTTGCCCT**
1251 **GCACCACCCAATCTGCCATTCCTGTCTAAATGTTTGTTTTGTCTATAATT**
1301 **CTTGCTGACTCATTCTAATGAGCTCAATTGTAACAAATTCTTTGTGTCCA**
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1851 CCATATCATTTGTCAAATTTGTCTCCACATGATAGTTTACTTTTGTTTAT
1901 GCAACGCGCATTTGGGCAACAAAAAGAAGCAAATCCTAATCTAGTGGCCA
1951 TTGGAAAGGAGATTGTGAAGAAATGTGGTGGTGTGCCTTTAGCAGCCAAG
2001 ACTCTTGGTGGTCTTTTACGCTTCAAGAGAGAAGAGAGTGAATGGGAACA
2051 TGTGAGAGATAATGAGATTTGGAGTCTGCCTCAAGATGAAAGTTCTATTT
2101 TGCCTGCTCTAAGACTGAGTTATCATCACCTTCCACTTGATTTGAGACAA
2151 TGCTTTGCGTATTGTGCAGTATTCCCAAAGGACACCAAAATGATAAAGGA
2201 AAATCTCATTACTCTCTGGATGGCGCATGGTTTTCTTTTATCAAAGGGAA
2251 ACTTGGAGCTAGAGGATGTGGGTAATGAAGTATGGAATGAATTATACTTG
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2351 GATACATGATCTAATCCATGATTTGGCTACATCTCTGTTTTCGGCAAGCG
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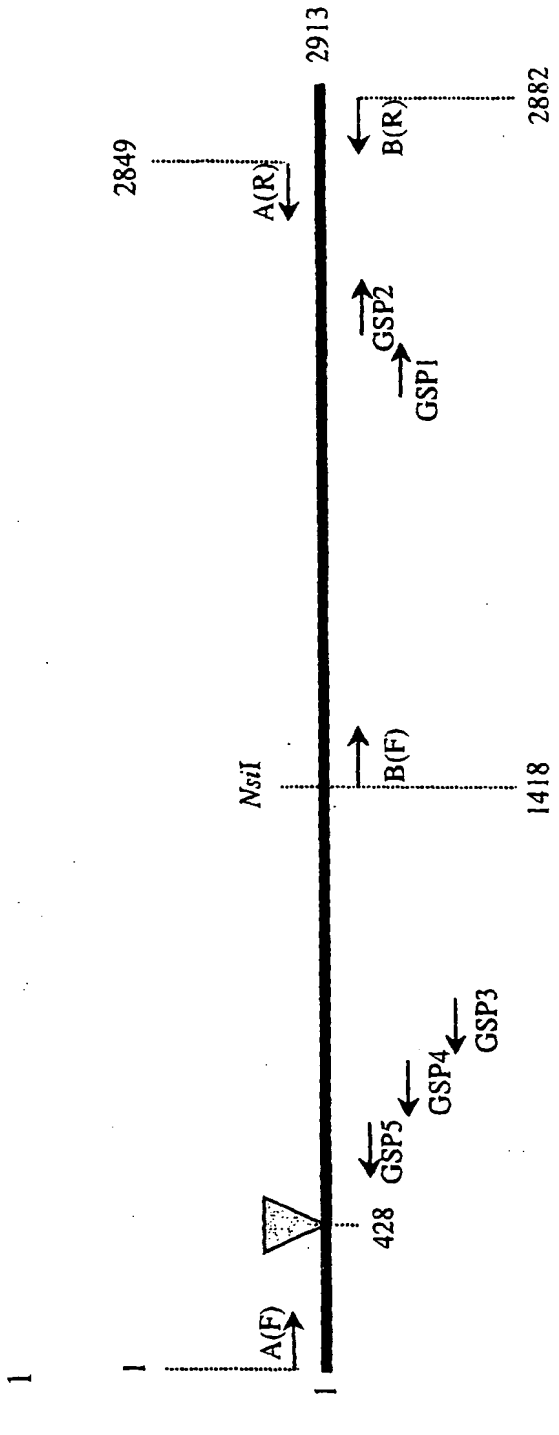

Figure 7

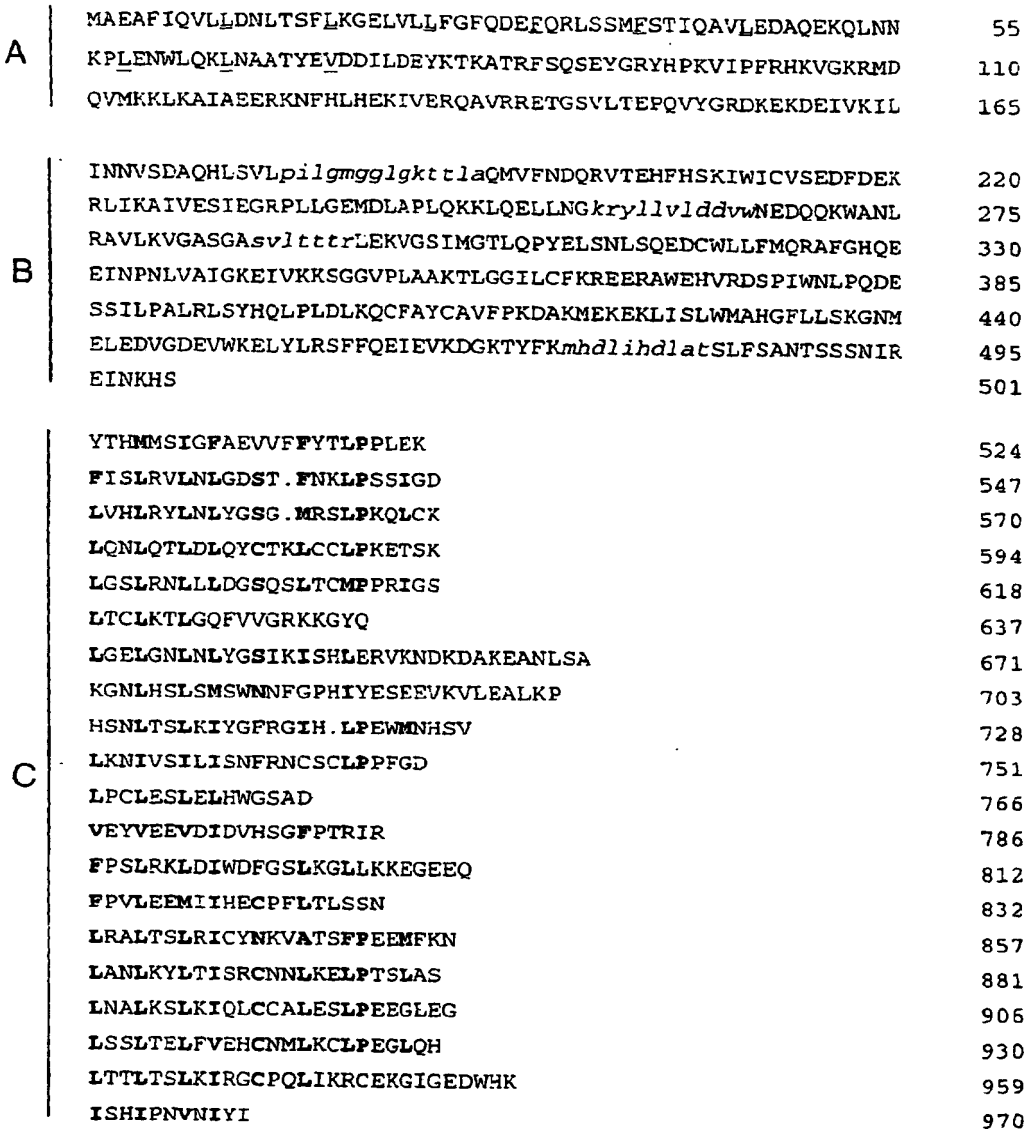

Figure 8

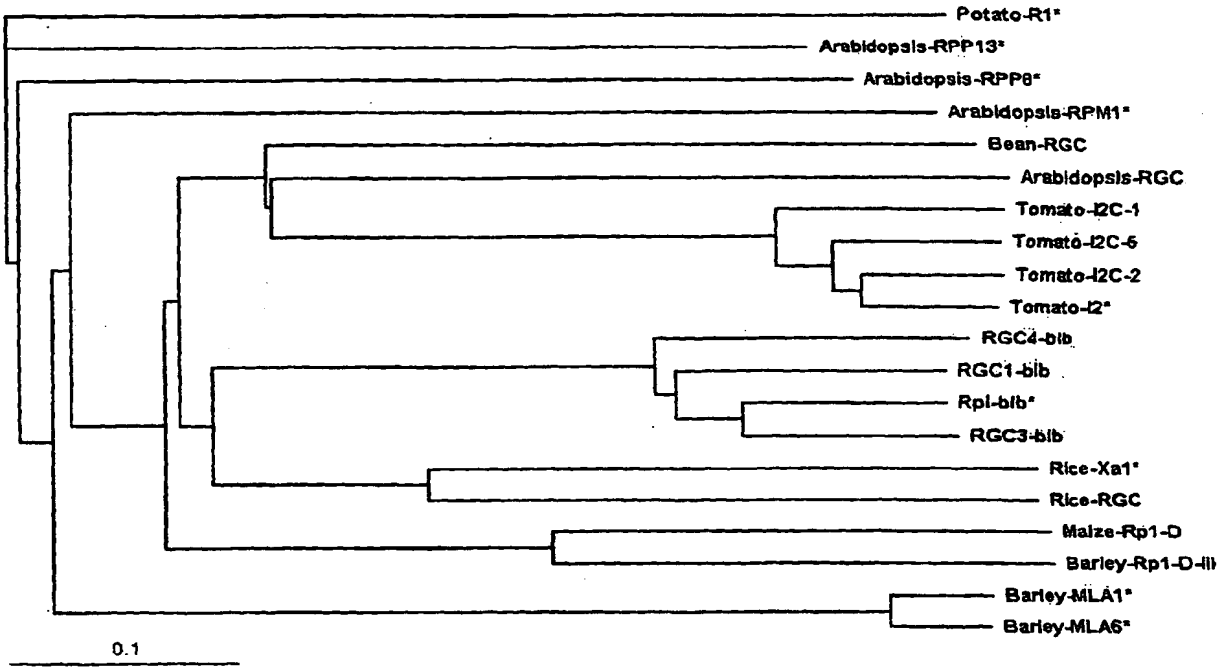

Figure 9

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Rpi-blb     MAEAFIQVLLDNLTSFLKGELVLLFGFQDEFQRLSSMFSTIQAVLEDAQEKQLNNKPLEN   60
RGC3-blb           V                                                D       60
RGC1-blb         L        F IQ   G V   EK  KK       M              KY AIK   60
RGC4-blb         L    E     IGDK   I   EK CEK   V        Q        KD AI    60

Rpi-blb     WLQKLNAATYEVDDILDEYKTKATRFSQSEYGRYHPKVIPFRHKVGKRMDQVMKKLKAIA   120
RGC3-blb                                 L                           N     120
RGC1-blb          V A         DC  E A  K AVL     RT T CY       KEM E  D     120
RGC4-blb          S A       G C NE I  E  RL F   GI N    I R  KEI E  D  S    120

Rpi-blb     EERKNFHLHEKIVERQAVR--RETG-----------------------------------   143
RGC3-blb           Q  I     AT--     -----------------------------------   143
RGC1-blb       R    D R I    A -- Q  -----------------------------------   143
RGC4-blb       RK  FL   T    AAAT   VGWQWGWARLEYKRLLLGVLMRIMSLRMHVSTCSTL   180

Rpi-blb     -----------------SVLTEPQVYGRDKEKDEIVKILINNVSDAQHLSVLpilgmggl   186
RGC3-blb    -----------------                                 K            186
RGC1-blb    -----------------F      K     E  E            YSEEVP          186
RGC4-blb    YEFKFYLCTPKVGARRCF      K        E            NV EE P F  I     240


Rpi-blb     gkttlaQMVFNDQRVTEHFHSKIWICVSEDFDEKRLIKAIVESIEGRPLLGEMDLAPLQK   246
RGC3-blb         S           R YP        D                  KS S-D        245
RGC1-blb                  I     NL   V   D                  KS G-D        245
RGC4-blb           I   E   K  NP   V   D          T IGN  - SSPHVE   SF     299

Rpi-blb     KLQELLNGkryllvlddvwNEDQQKWANLRAVLKVGASGAsvltttrLEKVGSIMGTLQP   306
RGC3-blb              F            H                  F                    305
RGC1-blb              F            E  D       I       I I       I        L 305
RGC4-blb                        D LE   K     T   R   I A                   359

Rpi-blb     YELSNLSQEDCWLLFMQRAFGHQEEINPNLVAIGKEIVKKSGgvplaaktlggILCFKRE   366
RGC3-blb           P    F  .                          C                R     365
RGC1-blb     Q             K    C  T TS K ME         C              L R     365
RGC4-blb     H      PH SL         Q K A               C              L R     419

Rpi-blb     ERAWEHVRDSPIWNLPQDESSILPALRLSYHQLPLDLKQCFAYCAVFPKDAKMEKEKLIS   426
RGC3-blb      E                                H      R   V         T  A  N  A  425
RGC1-blb      SE        E          N V         H      R             T I   Y  A  425
RGC4-blb      SE       NE  S                    H      R             T  I  N  T  479

Rpi-blb     LWMAHGFLLSKGNMELEDVGDEVWKELYLRSFFQEIEVKDGKTYFKmhdlihdlatSLFS   486
RGC3-blb    F             L       N   N              ES                     485
RGC1-blb         S                  N   N               S                  M  485
RGC4-blb                   L       N   N             A S N    I              539

Rpi-blb     ANTSSSNIREINKH----------SYTHMMSIGFAEVVFFYTLPPLEKFISLRVLNLGDS   536
RGC3-blb                 AN----------YDGY           SS SPSL Q  V        RN   535
RGC1-blb      SA  RS  Q  VKDDEDMMFIVTN KD       S   SS SPSLFKR V        SN   545
RGC4-blb      SA CG      VK----------D K TV      A  SS SPSL K  V        SY   589

Rpi-blb     TFNKLPSSIGDLVHLRYLNLYG-SGMRSLPKQLCKLQNLQTLDLQYCTKLCCLPKETSKL   595
RGC3-blb    NL Q                D S NFRI N   R              H  DS S     Q   595
RGC1-blb    E EQ     V          D S -NKIC    R              YN QS S     Q   604
RGC4-blb    KLEQ          L     D SC-NNF     ER             VHN YS N    Q   648

Rpi-blb     GSLRNLLLDGSQSLTCMPPRIGSLTCLKTLGQFVVGRKKGYQLGELGNLNLYGSIKISHL   655
RGC3-blb                -C   ST      L      S SC  I KR        K          S TK  654
RGC1-blb    C     V  H-CP  S         L        Y     ER        R     R A S T   663
RGC4-blb    S   H VV  -CP  ST        L        F I   S         K     C   S T   707

Rpi-blb     ERVKNDKDAKEANLSAKGNLHSLSMSWNNFGPHIYESEEVKVLEALKPHSNLTSLKIYGF   715
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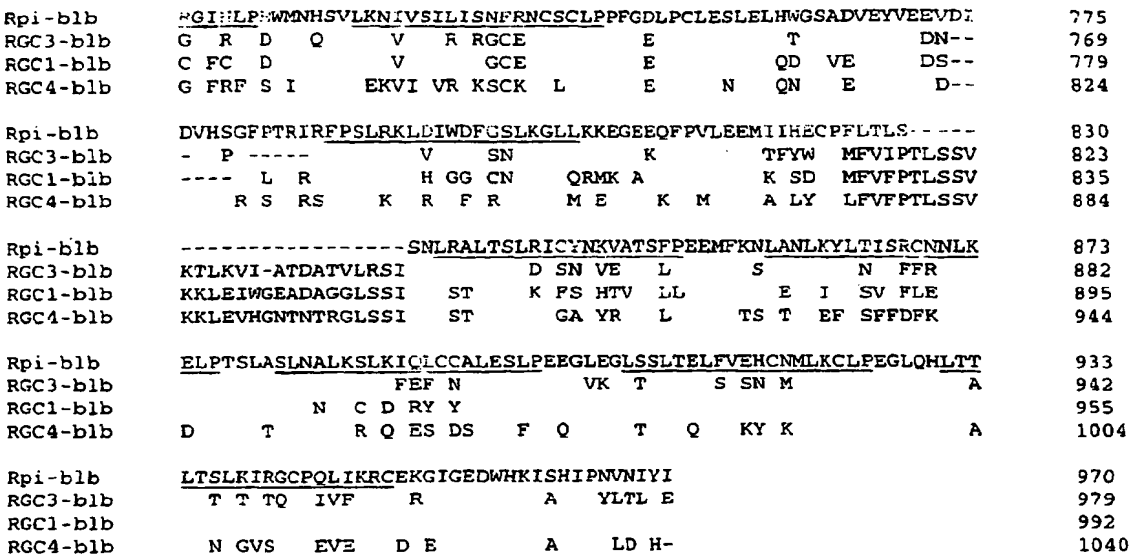

Figure 10

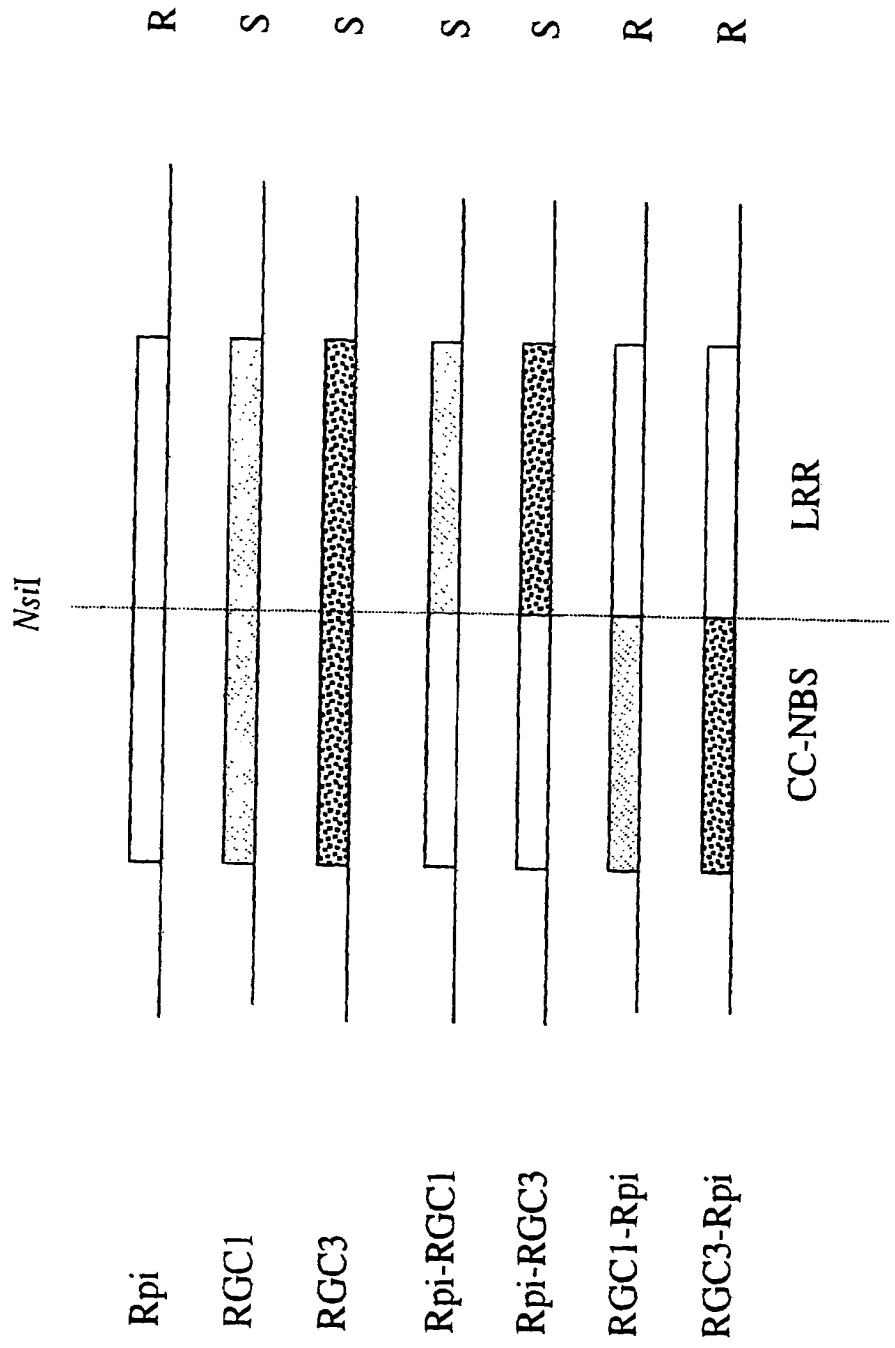

Figure 11

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 02 07 5565

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	DATABASE EMBL 'Online! EMBL; 5 September 2001 (2001-09-05) PAN Q. ET AL.: "Lycopersicon esculentum isolate Q194 nucleotide binding region of resistance-like gene, partial sequence" Database accession no. AF404480 XP002206417	1-23	C07K14/415 C12N15/82 C07K16/16 G01N33/50 C12N5/10 A01H1/04
Y	* abstract * -& PAN Q. ET AL.: "Comparative genetics of nucleotide binding site-leucin rich repeat resistance gene homologs in the genomes of two dicotyledons: tomato and arabidopsis" GENETICS, vol. 155, no. 1, 2000, pages 309-322, XP002207023	24-31	
X	DATABASE SWISSPROT 'Online! EBI; 1 December 2001 (2001-12-01) SASAKI T. ET AL.: "Putative NBS-LRR type resistance protein" Database accession no. Q94J89 XP002206418	1-23	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C12N C07K
Y	* abstract *	24-31	

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INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
MUNICH	22 July 2002	Marinoni, J-C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 02 07 5565

Claim(s) searched completely:
-

Claim(s) searched incompletely:
32

Reason for the limitation of the search (non-patentable invention(s)):

Article 53 (b) EPC - Plant variety

Further limitation of the search

Claim(s) searched completely:
-

Claim(s) searched incompletely:
1-32 all partially

Reason for the limitation of the search:

Present claims 1-4 and 6 relate to a nucleic acid defined by reference to a desirable characteristic or property, namely that it is identifiable by phylogenetic tree analysis as corresponding to the Rpi-blb, RGC1-blb, RGC2-blb and RGC4-blb gene cluster.
The claims cover all nucleic having this characteristic or property, whereas the application provides support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC for only a very limited number of such nucleic acids. Moreover, due to the purpose itself of the tree analysis, virtually any known late-blight LRR resistance gene, but also any gene whatever the extent of its homology to the genes of the invention and whatever its function, can be retrieved. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 84 EPC). This lack of clarity is such as to render a meaningful search over the whole of the claimed scope impossible. The additonal provisions of claims 2-4 and 6 are not considered as being true technical feature that would enable the skilled person to define properly the nucleic acids for which protection is sought (Article 84 EPC) and to put the invention into practice without undue burden (Article 83 EPC).

Additionally, claim 15 tries to define a proteinaceous substance by reference to a result to be achieved, namely that it provides at least partial resistance to oomycete infection, whereas only a very limited number of such proteinaceous substances has been disclosed in the application as filed.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the proteins having the sequences of SEQ ID No. 41 to 44 and the nucleic acids encoding them corresponding to the Rpi-blb,

European Patent Office

INCOMPLETE SEARCH
SHEET C

Application Number
EP 02 07 5565

RGC1-blb, RGC2-blb and RGC4-blb genes respectively, or fragments thereof, and to sequences sharing at least 50% identity with the sequences disclosed in the specification (see page 6, lines 5-8).

Consequently, all claims referring back directly or indirectly to the nucleic acids of claims 1-6 have also been searched partially.

Additionally again, claims 18 and 20 are directed to molecules binding either the proteins of claims 15-18 or the nucleic acid of claims 1-6, whereas the application provides support and disclosure only for a limited number of such molecules i.e. antibodies on one hand and primer/probes on the other hand. The search has been restricted to antibodies and primers/probes respectively. All claims referring back directly or indirectly to claims 18 and/or 20 were partially searched too.

Additionally again, claim 32 is directed to a plant that does not necessarily contain the gene(s) of the invention and could be a plant that has been obtained through traditional breeding methods, which are excluded from patentability under Article 53(b) EPC.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 02 07 5565

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	DATABASE EMBL 'Online! EMBL; 8 June 2001 (2001-06-08) BOUGRI O. ET AL.: "Generations of ESTs from dormant potato tubers" Database accession no. BG890602 XP002206419 * abstract *	1-23	
D,Y	VAN DER BIEZEN E A ET AL: "THE NB-ARC DOMAIN: A NOVEL SIGNALLING MOTIF SHARED BY PLANT RESISTANCE GENE PRODUCTS AND REGULATORS OF CELL DEATH IN ANIMALS" CURRENT BIOLOGY, CURRENT SCIENCE,, GB, vol. 8, no. 7, 26 March 1998 (1998-03-26), pages R226-R227, XP000924862 ISSN: 0960-9822 * the whole document *	1-31	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
Y	LEISTER D ET AL: "A PCR-BASED APPROACH FOR ISOLATING PATHOGEN RESISTANCE GENES FROM POTATO WITH POTENTIAL FOR WIDE APPLICATON IN PLANTS" NATURE GENETICS, NEW YORK, NY, US, vol. 14, December 1996 (1996-12), pages 421-429, XP000964717 ISSN: 1061-4036 * the whole document *	1-31	
A	VAN DER BIEZEN ERIC ET AL: "Plant disease-resistance proteins and the gene-for-gene concept" TIBS TRENDS IN BIOCHEMICAL SCIENCES, ELSEVIER PUBLICATION, CAMBRIDGE, EN, vol. 23, no. 12, December 1998 (1998-12), pages 454-456, XP002158209 ISSN: 0968-0004		
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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 02 07 5565

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	DONG F ET AL: "Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato." THEORETICAL AND APPLIED GENETICS, vol. 101, no. 7, November 2000 (2000-11), pages 1001-1007, XP001087853 ISSN: 0040-5752 ---		
A	ELLIS JEFF ET AL: "Structure, function and evolution of plant disease resistance genes." CURRENT OPINION IN PLANT BIOLOGY, vol. 3, no. 4, August 2000 (2000-08), pages 278-284, XP002206415 ISSN: 1369-5266 ---		TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	YOUNG NEVIN DALE: "The genetic architecture of resistance." CURRENT OPINION IN PLANT BIOLOGY, vol. 3, no. 4, August 2000 (2000-08), pages 285-290, XP002206416 ISSN: 1369-5266 ---		
A	OBERHAGEMANN P ET AL: "A GENETIC ANALYSIS OF QUANTITATIVE RESISTANCE TO LATE BLIGHT IN PATATO: TOWARDS MARKER-ASSISTED SELECTION" MOLECULAR BREEDING: NEW STRATEGIES IN PLANT IMPROVEMENT, KLUWER ACADEMIC PUBLISHERS, NL, vol. 5, no. 5, 1999, pages 399-415, XP001079515 ISSN: 1380-3743 ---		
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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 02 07 5565

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	THIEME R ET AL: "PRODUCTION OF SOMATIC HYBRIDS BETWEEN S.TUBEROSUM L. AND LATE BLIGHT RESISTANT MEXICAN WILD POTATO SPECIES" EUPHYTICA, KLUWER ACADEMIC PRESS, AMSTERDAM, NL, vol. 97, no. 2, 1997, pages 189-200, XP002912898 ISSN: 0014-2336 -----		TECHNICAL FIELDS SEARCHED (Int.Cl.7)

EPO FORM 1503 03.82 (P04C10)

European Patent Office

Application Number
EP 02 07 5565

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☒ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 02 07 5565

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-32 all partially

   The RPI-blb gene or RGC2-blb gene having the sequences of SEQ ID No. 35 and 36, the SEQ ID No. 37, all three sequences conferring late-blight resistance in Solanaceae, the protein encoded thereby having the sequence of SEQ ID No. 41, methods and plants related to said gene, etc...

2. Claims: 1-32 all partially

   The RGC1-blb, RGC3-blb and RGC4-blb genes of SEQ ID No. 38, 39, and 40, which do not confer late-blight resistance in Solanaceae, the protein encoded thereby having the sequences of SEQ ID No. 43, 42, and 44 respectively, methods and plants related to said gene, etc...